A LABORATORY MANUAL *for* ELEMENTARY ZOÖLOGY

HYMAN

Rachel Camp

Respiratory & Urogenital system
pp 128-130; mouth + Pharyngeal cavities

pp 4-5
Skeleton

Quiz Monday
1 digestive system
2 Respiratory
3 Buccal cavity
4 Urogenital system
5 ♀ & ♂
6 Skeleton

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A LABORATORY MANUAL FOR ELEMENTARY ZOÖLOGY

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A LABORATORY MANUAL
FOR
ELEMENTARY ZOÖLOGY

By
LIBBIE HENRIETTA HYMAN, PH.D.

THE UNIVERSITY OF CHICAGO PRESS
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INTRODUCTORY NOTE

This laboratory manual was prepared for the class in elementary zoölogy in the University of Chicago, and has been used in this course for some time. In the three months' time allotted for the course, nearly all of the work given in sections I to XII, inclusive, is completed, with the exception of the muscular and skeletal systems of the frog. The remaining sections have been added for publication because they seem to the author to be essential for a complete course in general zoölogy. The directions have been written entirely from the material, although, of course, many textbooks have been consulted throughout. An attempt has been made, not merely to give explicit directions for the study of the material, but also to point out the relation of each section of the work to the general principles of biology and to make clear why each particular kind of material has been selected. The sections are given, naturally, in the order which the author thinks most logical, but they can be shifted at the will of the instructor. Our experience, extending over many years of trial of both methods, has shown that starting the course with the dissection of a complex animal is much more satisfactory than introducing the student at the start to the lower invertebrates, for the reason that the simplicity of the latter cannot be appreciated and understood except with reference to completely differentiated animals. Any vertebrate would serve the purpose, but the arthropods are in general unsuitable because they introduce the problem of heteronomous segmentation for which the beginner is not prepared. Many teachers will object that the instructions are too detailed and that it is better pedagogy to compel the student to work out things by himself; but the large amount of ground which must be covered in a relatively short time and the impossibility of providing sufficient laboratory assistants for the large classes with which we have to deal necessitate detailed directions. The student pursuing the modern college curriculum simply does not have time to carry out an original investigation on the anatomy of an animal; and unless he is provided with detailed directions, instructors and assistants are overburdened with the task of explaining to him what he is looking at, what to do next, etc. There is no reason why this extra work cannot be avoided by including these explanations in the manual.

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PREFACE TO THE SECOND EDITION

This second edition has been entirely reset and for the most part rewritten. Chapter i has been considerably simplified and some of the material formerly in this chapter transferred to the chapter on the special anatomy of the frog. Additional explanatory matter has been introduced into chapter ii, in the hope of securing more satisfactory results from this chapter, for both at Chicago and elsewhere it has been found that students seem unable to comprehend the nature and purpose of an experiment. The chapter on mitosis has been completely revised and detailed directions given for additional materials. The account of the development of the frog has been extended to include all of the stages from hatching through metamorphosis. The chapter on ecology has been eliminated, most of the material formerly included there having been dispersed through other chapters. In response to a general demand, an account of Euglena has been introduced into the chapter on Protozoa and three new chapters have been added, one on the Mollusca, one on the Echinodermata, and one on a bony fish. An attempt has also been made wherever practical to add some suggestions for study and experiment on living material. Such work is, however, difficult to carry out with large classes. Other chapters not mentioned have not been altered to any great extent. A section on the activities of living insects planned for chapter xiv has been omitted because of lack of space.

The order of presentation of the subject has been retained for the most part, as not a sufficient number of users of the book desired an alteration of the order. I still retain my former opinion that the study of one of the more complex animals is the most satisfactory introduction to the subject. For those who prefer to begin with the one-celled animals and gradually proceed to the higher forms, the following order is suggested and has been found practical at the University of Chicago: chapter iii complete, viii, the experiment in vii is then begun, viA, ix to xiv, i, ii, iv, xv, viB, xvii, xviii.

I desire to express my thanks to those teachers who replied to my inquiries with suggestions for increasing the usefulness of the manual. They will find that many of their suggestions have been adopted.

L. H. H.

University of Chicago
April, 1926

GENERAL DIRECTIONS

1. Apply at Room 25 in the basement of the Physiology Building (Fifty-eighth Street near Ellis Avenue) for a microscope, presenting your tuition receipt. Ask for an ordinary compound microscope with high- and low-power dry objectives and two oculars. The rental charge, to be paid when securing the microscope, is two dollars per quarter. Procure a padlock (see 3), bring your microscope to the laboratory, and follow 4.

2. Supplies necessary for the course (see 3) are on sale at the biological storeroom in the basement of the Physiology Building (Fifty-eighth Street near Ellis Avenue). They are to be paid for in cash at the time of purchasing. There is no objection to the student's obtaining such supplies elsewhere if desired.

3. The following supplies are necessary:

One fine scissors.
Two small forceps with straight points.
One medium-sized scalpel.
One probe.
Two teasing needles.
One tripod hand lens.
Six slides.
One dozen ¾-inch square cover glasses.
Three medicine droppers.
A sheet of filter paper.
Package of lens paper.
A paper of pins.
A hand towel.
A padlock.
A laboratory coat or apron to protect the clothes is desirable.

4. Select a locker in the laboratory or have one assigned to you and place your padlock on the door. Keep your microscope, instruments, and other materials in this locker.

5. Purchase the following materials (University Press or other bookstore):

Drawing paper, No. 6—a smooth, stiff paper is required.
Note paper, No. 6.
Notebook cover, No. 6.
Hard drawing pencil, No. 6H (Venus, Kohinoor, etc., brands).
Eraser.
Ruler.
Pad of sand or carborundum paper for sharpening the pencil.

6. Select a place in the laboratory and retain this place throughout the course.

7. Present yourself with all of the foregoing materials ready for work at the begin-

ning of the first laboratory period. Do not handicap yourself at the start by failing to provide yourself with the necessary outfit.

8. A box of slides will be issued to each student. Examine them to see that none are damaged or broken except as already marked. These slides are to be returned at the end of the course and a charge will be made for each broken or injured slide. Students are requested to handle the slides with care. Do not stack them one on top of the other. Do not keep in a warm place as this will cause the mounting to run. Keep the box set on end as much as possible as this prevents running of the mounting or sliding of the specimens to one side.

General Instructions Regarding Drawings

1. *All drawings must be made on good quality drawing paper with a hard pencil. As experience has shown that hard pencil drawings are the most satisfactory, no other kind will be accepted.*

2. All drawings must be made from the actual material, unless otherwise directed, and completed in the laboratory. The making of rough sketches in the laboratory to be completed elsewhere is unscientific, inaccurate, and not permissible. Drawings copied from textbooks will not be accepted.

3. The drawing should contain all structures mentioned in the outlines, as only those readily found are called for. In case you cannot find any structures mentioned, ask the assistants to help you. Put into the drawing only what you have actually seen, and in case you were unable to find certain things make a note to that effect under the drawing.

4. The prime requisite of a drawing is accuracy, i.e., it must resemble the actual specimen before the student as closely as possible. Drawings are not to be diagrammatized unless the outlines expressly direct to make them so. Next to accuracy, neatness and good arrangement on the page are desirable.

5. Make your drawings large enough to show clearly all details asked for; students tend to make drawings too small. The more details called for, the larger the drawing must be.

6. Always keep the pencil sharp by means of sandpaper.

7. Draw on one face of the page only, on the face which lies to your right hand with your notebook open.

8. Label fully. Label everything in the drawing regardless of whether the same structures have already been labeled in some previous drawing. With a ruler draw a straight line out from the object to be labeled and write or print the label so that it is parallel to the top and bottom edges of the page.

9. Do not write notes on the drawings. Write them on separate pages and insert them next to the drawings to which they refer.

10. Students who declare that they "cannot draw" will receive little sympathy. Anyone can make the simple line drawings required in this work. To make a drawing proceed as follows. First determine how large it is to be and select a proper space on the page. Then rule this space with very light vertical and horizontal guide lines so that your drawings will be symmetrical. With a ruler further reduce or enlarge the length or

width of the actual object to fit the space selected. Then with very light lines make an outline of the object; then, constantly referring to the object, correct this with light lines until the proportions and details are as nearly like the object as you can possibly get them. Then erase the light lines until you can barely see them, and go over them making the final lines firm and clear. Every line upon the drawing must represent a structure actually present on the object. Avoid shading, color, crosshatching, etc.

Notes

1. Whenever desirable, notes are to accompany the drawings and are to be written on separate sheets which are to be inserted in the notebook adjacent to the sheet containing the drawings.

2. Avoid detailed notes containing material in the laboratory outlines.

3. The notes will consist of:

a) Answers to questions contained in the laboratory outlines unless such questions are obviously answered in the drawings.

b) Additional explanations, original observations, or comments upon any of the laboratory work as the student sees fit.

c) Complete accounts of experiments performed in the laboratory, whether individual or demonstration. This account should follow some such plan as this: (1) purpose of the experiment, (2) observations and results, (3) conclusions and explanations.

4. It is preferable but not compulsory that the notes be written in ink. Write on one side of the paper only.

Additional Miscellaneous Directions

1. The letter (*A*) signifies consult the laboratory instructor; (*R*), consult a textbook.

2. Right and left refer to the animal, not to the student, unless so stated.

3. All work is to be individual unless the supply of material is insufficient.

4. When handling small living animals with a dropper be sure that the dropper has never been used for chemicals. Have one dropper plainly marked which you use only for water or other harmless solutions. It has often happened that the entire supply of animals for the class has been killed because some careless student used a dropper which he had previously employed for chemicals.

5. In dropping a cover glass upon a slide, first put one edge of the cover glass against the slide until the liquid on the slide comes in contact with the cover glass, then lower the cover glass slowly. In this way, air bubbles under the cover glass are avoided.

6. Mount living animals in water. The water should be sufficient in amount to come to the edges of the cover glass but not enough to cause the cover glass to float about. Absorb extra water with a piece of filter paper. While examining living animals be careful that the water does not dry up. When this happens the animals will begin to slow down their movements, to flatten out, and finally to burst. Replenish the water from time to time by placing a drop in contact with the edge of the cover glass.

7. Living tissues of an animal must be mounted in fluid of the same osmotic pres-

sure as the fluids of the animal. In the case of the frog, this is a .6 per cent solution of common salt.

8. When dissecting, have the animal firmly fastened to the wax bottom of the dissecting tray with pins. Insert the pins obliquely, not vertically, so that they will not get in your way. Dissection is usually to be carried out under water. Dissect with blunt instruments, forceps, or probe, and have an instrument in each hand, one to hold the part, the other to dissect with. Never cut anything unless you are sure what you are cutting. Dissect lengthwise along blood vessels, nerves, tubes, etc., not across them.

9. In staining tissues, use a small amount of the stain. If the stain is applied before the cover glass is put on, drop a small drop of the stain on the tissue, then cover. If the stain is applied after the cover glass has been put on, place a drop of the stain in contact with one edge of the cover glass, apply a piece of filter paper to the opposite edge, and draw the stain under by suction. It generally requires a few minutes for a stain to act, especially when living animals are to be stained. If too much stain is used the whole structure becomes uniformly stained, so that the details are blotted out. In this case, make a new preparation or remove the excess stain by running a little dilute acid under the cover glass.

The Use of the Microscope

1. Parts of the microscope. Remove the instrument from the case. It consists of a horseshoe-shaped *base* from which arises a vertical *pillar* from which extends an *arm* supporting a vertical hollow tube. Between the pillar and the arm is an *inclination joint* by which the arm can be tilted toward the user. At the lower end of the tube is a *revolving nosepiece* into which the objectives fit. Below the nosepiece is a round or square *stage* with a central opening. Beneath this is a *condenser* and below the condenser a movable *mirror*. The mirror is used to throw light into the condenser. The condenser is a system of lenses for focusing the light upon the stage where the object to be examined is placed. The amount of light passing through the condenser is regulated by means of an *iris diaphragm* situated on the under side of the condenser. This diaphragm is composed of movable leaves operated by a button at its side. *Hold the lens in the top of the tube with one hand.* Then tilt the microscope so that you can see the diaphragm and note effect on the opening between the leaves of sliding the button back and forth. The mirror has two surfaces, one plane, the other concave. The concave surface is generally to be employed, especially with artificial light.

2. The adjustment screws. On the arm near the tube are certain *screw heads*, by means of which the tube is moved up and down. In the newer makes of microscopes there are two pairs of vertical screw heads, one larger than the other. In the older makes there is a pair of vertical screw heads and a single horizontal screw head. The larger pair in the first case and the vertical pair in the second constitute the *coarse adjustment* by means of which the tube is moved a considerable distance at each turn. The other screw heads operate the *fine adjustment* by means of which the tube moves slightly at each turn. Turn these screw heads and note effect on the tube. Observe that when the screw heads are turned away from you the tube moves down; when toward you, up.

3. The lenses. Vision in the microscope is accomplished by two lenses, the *objective,* placed at the bottom of the tube, and the *eyepiece* or *ocular,* placed at the top. Find on a shelf in the microscope box two cylindrical metal cases. Unscrew these and remove from them the *objectives.* They are of two sizes, high and low power, the low power one being the shorter and having the larger lens at its small end. Screw the objectives in place on the revolving nosepiece, holding each with both hands to avoid possibility of dropping. On another shelf in the microscope box find the two *eyepieces* or *oculars,* high and low power, the low power being the longer and with a larger lens at its upper end. Place the low-power eyepiece in the top of the tube.

4. To use the low power:

a) Swing the low-power objective into place at the lower end of the tube. A slight click indicates that it has stopped at the right place. Have the low-power eyepiece in the top of the tube.

b) While looking through the eyepiece, turn the mirror toward the light and continue to manipulate it until the field (circular area seen in the microscope) becomes suddenly bright.

c) Take a slide from your box and place some of the lettering on the label over the center of the opening in the stage.

d) While looking at the outside of the tube lower the tube by means of the coarse adjustment screw until the objective is close to the label.

e) While looking through the eyepiece slowly raise the tube by means of the coarse adjustment until the letters come into plain view. The object is now said to be *in focus.* Note the *distance* from the object at which the low power comes into focus.

f) Observe the nature of the image formed by the microscope. Are the letters right side up? How would you have to place them on the stage in order for them to appear right side up in the microscope? If you wish to move the image to the right or left or up or down in which directions must you move the slide?

5. To use the high power:

a) The object must always be under a cover glass when the high power is to be used.

b) The high power CANNOT be used with thick or thickly mounted or opaque objects.

c) The object to be viewed *must always be found first with the low power. Never* try to examine anything first with the high power.

d) Pull off a few threads from the torn edge of a piece of filter paper. Place on a clean slide and put a clean cover glass over them. Focus on them with the low power as directed above. Then place them in the exact center of the field as seen with the low power.

e) While continuing to look through the eyepiece swing the nosepiece around so that the high-power objective comes into place.

f) The object should be visible and should be nearly in focus. It is brought into exact focus by means of the fine adjustment screw. If the object is not visible when the

high power is swung into place, then the microscope is not perfectly adjusted and the following procedures must be followed to find out the difficulty:

(1) The center of the field of the high power may not be the same as the center of the field of the low power. Consequently as the high-power field is very much smaller than the low-power field, the object will not be in the field at all when the high power is swung around. The remedy is to get the object into the high-power field, then swing back to the low power. Note where the object is when viewed with the low power and remember to place the object in this spot instead of in the center of the low-power field when preparing to use the high power.

(2) When the high power is swung around the object may be in the field but is so far out of focus that it is not seen. This is the common difficulty. The remedy is to screw the tube considerably up or down after the high power is in place until a focus is obtained. You will have to find out by trial whether to screw up or down. If up, then always remember to raise the tube of the microscope before swinging the high power into place as otherwise the objective will strike against the slide.

(3) Have the laboratory instructor help you find out the peculiarities of your microscope.

g) Note how close to the object the high power focuses. This is one reason that thick objects cannot be viewed with the high power. Another reason is that a clear focus cannot be obtained on thick objects with high-power lenses. In using high powers care must always be taken not to run the objective into the slide as this will break the slide and may injure the lens.

6. Adjustment of the light. After getting a focus on an object with either low or high power, *always adjust the light* to the best advantage by means of the iris diaphragm. Students as a rule use too much light, which drowns out the details of the object and is hard on the eyes. *Always adjust the light for every object looked at* as the most favorable light is never the same for two different objects. Always readjust the light after changing to high power as the amount of light best for the high power is always different from that best for the low power. In examining transparent objects the light should be cut down by means of the diaphragm *before* trying to focus on the object for transparent objects are invisible in too much light.

7. Changing the focus. As the microscope is an optical instrument, the planes of focus of its lenses are geometrical planes, i. e., planes without thickness. All objects viewed through the microscope have an appreciable thickness. It is, therefore, obvious that *no object, no matter how thin it is,* can be seen in its totality in a *single plane of focus,* as some parts of the object are certain to lie outside that plane. *It is, therefore, essential to change the focus continually while viewing an object in the microscope.* This is particularly necessary when using high powers. *Students almost invariably make the grave mistake of getting an object in focus and then examining it without any further change of focus* with the result that they do not see all parts of the object, nor get a true idea of the relation in space of its parts. The practiced microscopist never takes his hand from the adjustment screw but continually changes the focus as he looks first at one part, then another. The student should at once form the habit of doing likewise.

8. Moving the slide. As the image in the microscope is reversed the slide must be moved in the opposite direction from that in which it is desired to move the image. This will soon become a habit. In moving the slide *do not put both hands* upon it but grasp it by the edges between the thumb and index finger of one hand. Learn to use one particular hand for moving the slide about while keeping the other hand on the adjustment screw.

9. The spring clips. Most microscopes are provided with a pair of clips which fit into holes on the stage and are intended to be used to hold the slide on the stage when tilting the tube by means of the inclination joint. The student is recommended not to use these clips. Although it is true that the tilted position of the tube is more comfortable to the observer, the position is disadvantageous because the slide usually has to be moved about a great deal and the clips must be removed and replaced each time. The continual snapping of the clips on the slide is very likely to injure the slide. Further, only permanently mounted slides can be examined in the tilted position. It is, therefore, desirable for the student to learn at once to use the microscope in the vertical position.

10. Miscellaneous directions:

a) If the image is dim or indistinct or if the field rolls when the focus is changed, or if the high power cannot be brought to clear focus, then in all probability the objective (sometimes the eyepiece) is wet or dirty or has become clouded with your breath. Clean the lenses with lens paper. If any balsam (sticky material with which permanent mounts are made) gets on the objective, have the laboratory instructor clean it off for you. Difficulty in getting a clear focus with the high power may be due to too great thickness in the object being examined. The object should then be made thinner. If these measures fail to yield a clear focus with the high power, have laboratory instructor examine the surface of the objective for scratches or dents. It may be necessary to exchange the high-power lens for a better one (Room 2, Press Building). In case of any trouble with the microscope, do not tamper with the instrument but call the laboratory instructor.

b) If images of buildings, etc., appear in the field, they may be obliterated by using the concave surface of the mirror, or by lowering the condenser.

c) In working with artificial light, use the concave surface of the mirror.

d) Round black rings in the field are air bubbles under the cover glass.

e) If the fine adjustment screw head turns without producing any effect upon the tube it has come to the upper limit of its range. Raise the tube by means of the coarse adjustment and then screw the fine adjustment down. If the fine adjustment will not turn at all it has come to the lower limit of its range and must be screwed up.

f) Have your stool at just the right height so that you can just reach the eyepiece comfortably with your eye. Sit squarely at the microscope. Failure to observe these rules will result in a surprising amount of fatigue. Have the instrument facing directly away from you. Do not turn it sidewise; turn the mirror, not the microscope, toward the light. Students who wear glasses regularly should not remove them when using the

microscope. With a little practice one can learn to use the ordinary monocular microscope with glasses on.

g) Keep the microscope clean and free from dust. Do not let it stand in the sunlight.

h) *Do not use sunlight for illumination. A north or east (after noon) light is best.*

i) Keep both eyes open when looking through the microscope. If you find this difficult try placing an oblong of stiff green paper around the top of the microscope so that the unused eye will not see objects.

j) The magnification of the low-power objective when combined with the low-power ocular is about 50; of the high-power with high-power ocular about 500.

KEEP THE LENSES CLEAN
ADJUST THE LIGHT FOR EVERY OBJECT
ADJUST THE FOCUS CONTINUALLY WHILE YOU LOOK

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Thursday } Read p. 1–8

(p 41–3)

I. GENERAL SURVEY OF AN ANIMAL

It is believed that the best introduction to the science of zoölogy is the thorough study of a single, relatively complex animal. For this reason, we shall first examine the anatomy, the physiology, and the microscopic structure of one of the commonest animals, the frog. After learning how such an animal is constructed and how it employs the structures which it possesses, we shall be in a position to understand the construction of other animals and to appreciate by what changes and alterations animals have been built up from a simple starting point.

A. EXTERNAL ANATOMY OF THE FROG

There are four kinds (*species*) of frogs in the United States common enough and large enough to serve for laboratory study. These are the bullfrog, the green frog, the leopard or grass frog, and the pickerel frog. These four species differ slightly in minor points of anatomy. The bullfrog (*Rana catesbiana*) may reach a length of seven to eight inches and is of a general olive or brown color above, often with irregular darker mottlings. The green frog (*Rana clamitans*) is similar in color but smaller (three to five inches). The leopard frog (*Rana pipiens*) and the pickerel frog (*Rana palustris*) are two to four inches long with numerous conspicuous black spots which are edged with light yellow in the leopard frog, not so edged in the pickerel frog. The bullfrog and leopard frog are most commonly used in zoölogical courses. Other differences between these species will appear later.

The *external anatomy* is best studied on the dead frog. The frog may be killed with ether or chloroform or by pithing the nervous system (see Appendix). After either method, many activities continue for a considerable length of time. The heart keeps on beating and parts may be caused to move by applying an electric current or other stimulus to the proper places. Is the frog dead? What is meant by death in the higher animals? Do all parts of an animal die at the same time? What part of the frog is really dead?

Obtain a dead frog, place it in a dissecting pan, and carefully note the following points. The body of the animal consists of a flattened *head*, triangular in outline, and a short stout *trunk;* there is neither *neck* nor *tail*. Two pairs of *limbs* or *appendages* project from the trunk; they are named the *fore* and *hind limbs*. Head and trunk (also neck and tail when present) constitute the *axial part* of an animal while the limbs or appendages and their supports are designated the *appendicular part*. The head is the *anterior* end; the opposite region the *posterior* end; and these terms are also employed to indicate the relative positions of other structures with reference to one another, as, for instance, one would say that the fore limbs are anterior to the hind limbs. The back or upper surface is the *dorsal* side; the lower or under surface, the *ventral* side. Central portions of the animal are designated as *median;* those toward the sides or margins as *lateral*. Parts at or near the surface are spoken of as *peripheral* or *superficial* or *exter-*

nal; those situated more in the interior as *central* or *deep* or *internal*. All of these terms are also used to indicate relative positions of parts.

Nearly all animals exhibit the property of *symmetry*, that is, their parts are arranged and spaced so as to balance one another. Several kinds of such balanced arrangements of parts occur among animals and are known as *types of symmetry*. These types of symmetry are defined by means of certain imaginary lines, planes, and axes, called *planes* and *axes of symmetry*. An imaginary line passing along the middle of the dorsal surface from anterior to posterior end is the *median dorsal line;* a similar line along the middle of the ventral surface is the *median ventral line*. A plane passing through these two lines from anterior to posterior end and dorsal to ventral surface is the *median sagittal plane*. An imaginary line in the median sagittal plane from anterior to posterior end is the *antero-posterior axis*. Other planes often referred to are the *horizontal* or *frontal* plane or section, which is any plane parallel to dorsal and ventral surfaces; and the *transverse* or *cross* plane or section, which cuts vertically across the body at right angles to dorsal and ventral surfaces. An animal so constructed that there is but *one* plane—namely the median sagittal plane—which will divide it into *right* and *left halves* that are identical or nearly so except that one is the reverse of the other, is said to be *bilaterally symmetrical;* and this kind of symmetry is called *bilateral symmetry*. Is the frog bilaterally symmetrical so far as you can observe externally? Is man? The majority of animals are bilaterally symmetrical. Organs or parts which are cut in two by the median sagittal plane are spoken of as *median* or *unpaired* organs; parts placed symmetrically to each side of this plane are called *paired*. Thus the tail of an animal is unpaired, the limbs paired.

Returning now to the study of the external anatomy of the frog note differences between dorsal and ventral surfaces as to color and color pattern, structures present, thickenings of the skin, etc. Compare color of different individuals and if available, different species. Can you suggest any reason for the greater development of color on the dorsal surface? Frogs can alter their color considerably (*R*).

Study the *head*. It possesses a number of distinctive features which are generally throughout the animal kingdom associated with the anterior end. These structures are located chiefly on the dorsal surface. Near the anterior end of this surface are two small openings, the *nostrils* or *external nares*. These, unlike our own, can be opened and closed by lowering and elevating certain bones at the tip of the upper jaw. Posterior to the nares are the large prominent *eyes* in which may be distinguished (best in living specimens) the *iris*, flecked with gold, surrounding a central dark opening, the *pupil*. Each eye is provided with *upper* and *lower eyelids*, folds of skin above and below the *eyeball;* and a *third* eyelid, the *nictitating membrane*, a thin, translucent membrane, which is generally drawn partly or completely over the eyeball in dead frogs. It is really an outgrowth of the lower lid. The nictitating membrane is represented in our own eyes by a small crescent-shaped fold near the inner corner (semilunar fold).

Obtain a living frog, gently touch an eyeball and observe the action of the eyelids. Which eyelids are movable? Touch the eyeball more forcibly. What happens? The cavity in the skull which holds the eyeball is designated as the *orbit*. In the frog unlike ourselves it is open below, thus permitting the action noted.

Returning now to the dead specimen, note a circular or oval area of tense skin just posterior to the eye. This is the *eardrum* or *tympanic membrane*, which covers the cavity of the *middle ear*. The eardrum is about as large as the eye in most species of frogs but in the males of some species (e.g., the bullfrog) it is considerably larger than the eye. Near the center of the eardrum a small elevation may usually be noticed; this marks the place where the outer end of a small bone, the *columella*, presses against the internal surface of the drum. This bone transmits inwardly the vibrations of the drum. The *external ear*, and passage leading in from it, prominent in ourselves, are entirely wanting in the frog.

In the median dorsal line of the head, slightly anterior to the level of the eyes, a small light-colored spot, the *brow spot*, may usually be found; it is difficult to see in dark individuals. This spot represents the location of an eye, formerly present in vertebrates.

Around the margin of the head runs a slit, the *mouth opening*, having the form of a parabola. It is bounded above by the *upper jaw*, below by the *lower jaw;* these jaws are supported by bone as may be proved by feeling them. The upper jaw is immovably fastened to the *skull* (bony support of the head), while the lower jaw can be moved up and down by means of a hinge joint at the posterior end of the mouth slit, below the eardrum. This meeting place of upper and lower jaws is called the *angle of the jaws*.

The *trunk* presents few features. There are generally two light-colored ridges extending posteriorly from the eyes along the lateral regions of the dorsal surface of the trunk. They are called the *dorso-lateral dermal plicae* or *folds* and represent thickenings of the skin caused by the presence of large poison glands in the skin. The dermal plicae are absent in the bullfrog. At the posterior end of the trunk on the dorsal side between the bases of the hind legs is a small opening, the *anus* or *vent*, which is the end of the digestive tract. In the middle of the back a characteristic hump, the *sacral hump*, best observed in the sitting live frog, is present, owing to certain features of the skeleton at this place (see a skeleton of the frog).

The *fore limb* is short and consists of three divisions: *upper arm* or ~~*brachium*~~, *forearm* or ~~*antibrachium*~~, and *hand* or ~~*manus*~~. The hand is again divisible into three parts: *wrist* or *carpus* (not externally noticeable in the frog), *palm* or *metacarpus*, and *fingers* or *digits*. How many fingers has the hand? To which of your fingers do these correspond (*R*)? The rudiment of the missing finger may be felt under the skin at the base of the present first finger and may be seen on the skeleton of the hand. In the male frog, the inner finger or "thumb" is enlarged and swollen at the base especially in the breeding season. The forearm of males is also said to be stouter and stronger than in females. On the ventral surface of the hand note padlike thickenings, the *subarticular pads*. Can you suggest a function for them?

The hind limb likewise consists of three parts: the *thigh* or *femur*, *shank* or *crus*, and *foot* or *pes*. The foot is again divisible into *ankle* or *tarsus*, *sole* or *metatarsus*, and *toes* or *digits*. The ankle is unusually long in the frog. Why? There are five toes, the smallest corresponding to our great toe. Note lengths of the other four. At the inner side of the first toe is a rudimentary sixth toe, the *prehallux*, which may be felt on the specimen or seen on a skeleton. Such additional digits are not uncommon in aquatic

vertebrates and seem to serve the purpose of producing a larger swimming surface. Observe the *web* connecting the toes and used in swimming; its extent differs in different species of frogs. ~~Note *subarticular pads*.~~

Frogs do not possess any *external genitalia* (sex organs) and in many species it is difficult or impossible to determine the sex except by dissection. In the bullfrog and the green frog the eardrum is much larger than the eye in males, of the same size as the eye in females. In many species there are more or less definite differences in color or color pattern between the sexes. The females are frequently larger than the males. During the breeding season the base of the first finger is swollen and thickened in males forming the *thumb pad* but this difference between the sexes is generally not very apparent. In our common leopard frog the only external difference between the sexes is the larger size of the female.

The skin of frogs is mostly smooth but may bear warts or thickenings and is slimy in life. There are no hairs, scales, or claws. Note that in general the skin is more loosely attached to the body than in most animals.

Make an accurate drawing of the frog from the dorsal side, putting in all of the structures and parts which have been mentioned, but omitting color pattern. Before beginning to draw, *reread carefully* the directions about drawings. *Label* this and all subsequent drawings fully.

NOTE.—At the close of the first laboratory period, make an incision about one-half inch long through the skin only of the left abdominal wall of your frog and place the animal in the jar of preserving fluid which will be assigned to you. The animal must be kept in this jar when not in use. It must *never* be left out on the tables and *never* allowed to become dry.

B. GENERAL INTERNAL FEATURES

1. The mouth and pharyngeal cavities.—In the head inclosed by the jaws is a large cavity. Open the mouth to its widest extent, cutting through the angle of the jaws if necessary (necessary in preserved specimens). The anterior part of the large cavity thus revealed is the *mouth* or *oral* or *buccal cavity*, the posterior part the *pharyngeal cavity;* but no very definite boundary between these parts can be assigned.

a) *Roof:* The projecting margin of the upper jaw is called the *upper lip fold*. Internal to this fold and more or less concealed by it is a semicircular row of *teeth*, the *maxillary teeth*, borne on certain bones of the upper jaw. Pull the lip fold outward in order to see the teeth more clearly. Internal to the teeth is a deep groove, the *sulcus marginalis*. At the tip of the jaw this groove deepens into a pit, the *median subrostral fossa* or *pit;* on each side of this is an elevation, the *subrostral pulvinar* or *cushion;* and to the side of each cushion is another depression, the *lateral subrostral fossa*. The roof of the mouth cavity may be defined as the part bounded by the sulcus marginalis.

At the anterior end of the roof are two oval openings, the *internal nares or choanae*, which are the internal openings of the nasal cavities. Prove this by passing a bristle into one of the external nares. The bristle is felt to enter a chamber, the *nasal cavity*, and emerges through the internal naris. The external nares, nasal cavities, and internal nares constitute a pair of *respiratory passages* through which air is drawn into the mouth cavity. Between the internal nares are two rounded projections composed of

teeth, called the *vomerine* teeth from the name (*vomer*) of the bones which bear them. The greater part of the roof of the mouth cavity is occupied by two large rounded prominences caused by the eyes. Recall that the eyes can be withdrawn into the mouth cavity.

In the roof of the pharyngeal cavity on each side near the cut jaw bone is a conspicuous opening, the beginning of the *auditory* (or *Eustachian*) tube. Pass a probe into the opening and note that the probe is stopped by the eardrum. The auditory tube is thus a passage extending from the pharyngeal cavity into a part of the ear called the *tympanic cavity* or *cavity of the middle ear*, which is covered externally by the eardrum. The purpose of this arrangement is the adjustment of air pressure in the ear.

Make a drawing of the roof of the mouth and pharyngeal cavities.

b) *Floor:* The edge of the lower jaw forms a ridge which fits into the sulcus marginalis. At the tip of the lower jaw is an elevation, the *prelingual tubercle*, and on each side of this a depression, the *prelingual fossa*. Note how exactly these fit into the elevations and depressions of the upper jaw. Are teeth present on the lower jaw? The greater part of the floor of the mouth cavity is occupied by the *tongue*. Note the size, shape, and attachment of this organ and find out how it is used in catching prey (*R*). Turn the tongue forward and feel the floor behind the tongue. The floor is stiffened by a cartilaginous plate, part of the *hyoid apparatus*, a portion of the skeleton.

The remainder of the floor of the cavity may be regarded as belonging to the pharynx. In the median line behind the tongue is a circular hardened elevation, the *laryngeal prominence*, which bears in its center an elongated slit, the *glottis*. The glottis is the entrance to the lungs. In males, a slit is present to each side of the glottis near the edge of the lower jaw. This is the entrance to the *vocal sac*, a pouch which extends backward under the skin behind the eardrums and can be greatly expanded in some species, as the leopard frog. The vocal sacs act as resonators increasing the volume of sound in croaking.

At the back of the pharynx, roof and floor converge to a large opening, the *esophagus*, into which food passes from the pharyngeal cavity.

Draw the floor of the mouth and pharyngeal cavities.

2. The layers of the body wall.—The trunk of the frog, as we shall soon see, is hollow. It thus consists of an external coat, or wall, the *body wall*, and an internal cavity, the *body cavity* or *coelome*. The body wall is composed of several layers, of which the skin constitutes the outermost. The general features of the skin have already been noted.

Remove the skin slowly from the trunk of the frog by making a long slit in it and peeling it off. In doing so note the following structures: *blood vessels*, dark-colored tubes, extending to the under surface of the skin; *nerves*, elastic white fibers proceeding to the skin, especially in the mid-dorsal region; and the partitions composed of a weblike material, the *subcutaneous connective tissue*, by means of which the skin is attached at intervals to the underlying flesh. The space beneath the skin is thus divided by these partitions into compartments, the *subcutaneous lymph spaces* or *lymph sacs*, which in life are filled with a fluid, the *lymph*. The lymph is a colorless fluid similar in composition to the blood. It bathes all parts of the body. The frog and its relatives dif-

fer from other vertebrates in this enormous development of huge lymph spaces not only under the skin but also throughout the body. This structural feature is probably associated with the amphibious habits of these animals.

The extensive supply of blood vessels to the skin should be noted. It is due to the respiratory function of the skin, to be discussed more fully later.

The removal of the skin exposes the next layer of the body wall, composed of *muscles* (flesh) and the *skeleton* which is imbedded in them and to which they are attached. The principal parts of the skeleton and some of the more conspicuous muscles will be identified.

The skeleton is the supporting framework of the body. It is composed of hard material, chiefly bone, with some cartilaginous portions. As the parts are mentioned locate them by feeling in the places indicated. A prepared skeleton should be on hand for comparison. The head contains a bony case, the *skull,* to which the *upper jaw* is immovably fused, the *lower jaw* hinged. An arch-shaped structure, the *pectoral girdle,* partly bone and partly cartilaginous, is situated in the anterior part of the trunk at the level of the fore limbs. The dorsal ends of the girdle are seen on each side of the mid-dorsal line as a flat thin bone, the *suprascapula,* with a cartilaginous border. In the median ventral line in the anterior half of the trunk, an elongated hard structure can be felt and indistinctly seen. This is the *sternum* or *breastbone,* composed of several bones and cartilages. Its anterior and posterior rounded cartilages are more clearly visible than its middle part. The pectoral girdle is attached to the sternum on each side and both of these structures support the anterior part of the trunk and the fore limbs. The posterior part of the trunk and the hind limbs are supported by a bony structure, the *pelvic girdle,* shaped like a **U**. Part of it can be felt on both dorsal and ventral sides at the base of the hind legs. The rest of it extends forward on each side of the posterior half of the back as a hard ridge, the *ilium* bone. The mid-dorsal line is depressed, forming a groove. Pass an instrument along this groove and feel the hard structure, the *vertebral column,* which underlies it. On a prepared skeleton note that the vertebral column consists of a longitudinal series (nine in the frog) of bony ring-shaped structures, the *vertebrae,* with projecting *transverse processes.* Returning to your specimen, note the enlarged ends of the transverse processes of the ninth vertebra, to which the anterior ends of the ilium bones are firmly fastened. The pelvic girdle is thus supported by the ninth vertebra which for this reason is known as the *sacral vertebra* or *sacrum.* This vertebra produces the *sacral hump* already noted. Posterior to the sacral vertebra the vertebral column terminates in a long slender bone, the *urostyle,* composed of several fused vertebrae. In the interior of each limb is a series of bones. All of these features of the skeleton except the urostyle are found throughout the vertebrates, including man. The absence of a tail in the frog renders the posterior part of the vertebral column somewhat atypical.

The *muscular coat* of the body wall consists of a large number of separate *muscles,* fleshy masses of various shapes, which are attached directly or indirectly to the skeleton and accomplish all movements of head, trunk, and limbs. Only a few of the more conspicuous external trunk muscles will be noted here. Observe that the lateral and ventral abdominal walls are supported by muscles only, skeleton being absent. The

lateral walls of the trunk are formed superficially by the *external oblique* muscles. The median ventral line from the posterior end of the sternum to the pelvic girdle is occupied by a white strip, the *linea alba*. At each side of this runs an elongated muscle, the *rectus abdominis*. Several muscles of the anterior part of the trunk which converge toward the upper arm are named collectively the *pectoral* muscles. A flat thin muscle, the *mylohyoid*, extends transversely across the ventral surface of the lower jaw. On the dorsal surface the most conspicuous muscles are those of the shoulder region, used in operating the fore limbs. Masses of muscle occur alongside the vertebral column. The arrangement of muscles seen in the frog is very similar to that of vertebrates in general, including man.

Make a longitudinal slit through the muscles on the ventral side to the left (frog's left) of the linea alba. Spread open the walls and note that you are looking into a large cavity, the *body cavity* or *coelome*. Examine the internal surface of the muscular layer and observe that it is clothed with a smooth shining membrane. This membrane is the lining of the coelome and is called the *peritoneum*.

The body wall thus consists of three layers: the skin, the muscles with their contained skeleton, and the peritoneum.

3. **The viscera.**—The internal organs of an animal are known as *viscera* (singular, *viscus*).

Extend the cut just made from a point in front of the pelvic girdle up to the mylohyoid muscle, cutting through the bones of the pectoral girdle, keeping always slightly to the left of the linea alba. Make a cross-cut in the left wall just behind the fore limb and another just in front of the base of the hind limb to enable you to spread the edges of the cut more widely. Do not cut across the linea alba at present. Spread the cut edges as far as possible and look inside. A number of organs are immediately noticed. The more conspicuous ones will be identified at this point.

If the animal is a mature female, a large black and white mass will be seen nearly filling the coelome. This is the left *ovary*. The white coiled tube seen to the side of or dorsal to the ovary is an *oviduct*. In immature females and in males, the coils of the *intestine* are seen in the posterior part of the coelome. In mature females the intestine is more or less concealed by the ovaries. The large dark-red or brown lobed mass seen in the anterior part of the coelome is the *liver*. Protruding from under the left side of the liver or revealed by lifting this side is a cylindrical white tube, the *stomach*. In the median ventral region, just under the sternum, and lying in a depression in the anterior surface of the liver is the *heart*, inclosed in a delicate membrane, the *pericardial sac*. In recently killed specimens, the heart may still be beating. If the lungs happen to be inflated, the left *lung* is noticeable as a hollow spongy sac to the side of the anterior part of the liver. If the lungs are collapsed they are not noticeable. A branching yellow mass, the *fat body*, fastened to the dorsal wall at about the middle of the body, may be conspicuous in some specimens. At the posterior end of the coelome, a thin-walled sac, the *urinary bladder*, which may be inflated but which is usually collapsed and shriveled, is present.

In the median ventral line directly internal to the linea alba runs a conspicuous blood vessel, the *ventral abdominal vein*. Observe that this vein passes into the liver

by means of a membrane which supports it and also attaches the liver to the mid-ventral line. This membrane is the *falciform ligament of the liver*. It extends forward, attaching the pericardial sac to the mid-ventral line internal to the sternum.

After observing the relations of the ventral abdominal vein, cut transversely through the right abdominal wall behind the fore limb and in front of the hind limb. Pin out the flaps of skin on both sides to the bottom of a dissecting pan. In mature females remove the left ovary and oviduct by cutting through their attachments to the dorsal body wall.

4. **The coelome and the mesenteries.**—The *coelome* or *body cavity* is the space between the body wall and the viscera. In the frog it is divided into two compartments, the large general space already noted, which is named the *pleuroperitoneal cavity*, and the small space, the *pericardial cavity*, around the heart. Note again that the heart is inclosed in a delicate sac, the pericardial sac, which separates the heart from the rest of the viscera. Observe that the heart is freely movable within the sac, being in fact separated from the sac by a narrow space, the *pericardial cavity*, a portion of the coelome. We may here recall that in our own bodies there is a transverse partition, the *diaphragm*, across the middle. The diaphragm divides the coelome into anterior cavities, the chest or *pleural* cavities, containing the lungs, and a posterior cavity, the *abdominal* or *peritoneal* cavity, containing most of the viscera. But in the frog and other lower vertebrates, a diaphragm has not yet evolved, and one continuous cavity, called the pleuroperitoneal cavity, since it comprises the pleural and peritoneal cavities of higher vertebrates, is present.

The pleuroperitoneal cavity is lined by a smooth shining membrane, the *pleuroperitoneum*, more briefly but less correctly called the *peritoneum*. The pericardial cavity is lined by the *pericardium* which may be regarded (although not quite correctly so) as constituting the pericardial sac. These membranes not only line the coelomic cavities but form the outer coat of and support the viscera contained in these cavities. Pick up any of the viscera such as the liver or stomach and note that they are fastened at some point or points to the body wall or to other viscera by means of delicate membranes called *mesenteries* or *ligaments*. Thus, as already noted, the liver is so fastened to the mid-ventral line by the falciform ligament. A ligament or mesentery may be regarded as formed in the following way. Imagine the coelomic lining to leave the body wall opposite the organ, to pass over the surface of the organ, and to return to the body wall at the same point from which it left. We thus have produced between the organ and the body wall a double-walled membrane, composed of the coelomic lining: this membrane is the *ligament* or *mesentery* of that particular organ. The part of the coelomic lining which clothes the wall of the coelomic cavity is called the *parietal* peritoneum or *parietal* pericardium, etc., as the case may be; the part which forms the outer coat of the organs (to whose surfaces it is so tightly applied as to be inseparable from them) is named the *visceral* peritoneum or *visceral* pericardium, etc., or *serosa*. These relations of coelome and organs are common to all animals that possess a coelome and are, in fact, the unavoidable consequence of the coelomate condition.

The mesenteries and ligaments constitute an ingenious device which permits the viscera to adjust themselves to movements of the body and to carry out their own move-

ments and yet at the same time holds them in place with reference to each other and to the body wall.

C. THE SYSTEMS OF THE FROG

The frog and most other animals are made up of a number of definite structures, called *organs*, each of which performs a definite function. All of the organs which aid in performing the same function are considered as constituting a *system* or *tract*. In general in a complex animal there are ten systems: *integumentary* (skin and derivatives), *skeletal, muscular, digestive, respiratory, circulatory, excretory, reproductive, nervous,* and *sensory* systems. To this list there should probably be added in the case of vertebrates, an eleventh, the *endocrine* system, composed of a number of glands, called the *glands of internal secretion,* which were originally connected with other systems, but have lost such connection and have taken on general functions.

The service rendered to the animal by each of these systems, that is, its *function,* may be briefly stated at this point. The skin protects the surface of the body and in many animals assists the respiratory system. The skeleton supports the soft parts. Muscles constitute the mechanism of movement. The digestive system and its glands serve for the ingestion and digestion of food and the extrusion of indigestible matter. By means of the circulatory system substances are distributed throughout and collected from all parts of the body. The excretory system concentrates certain dissolved waste matters produced by the body and discharges them to the exterior. The reproductive system forms the male and female sex cells and various accessory fluids, all serving the end of reproducing the species. The nervous system controls and directs the activities of the animal both internal and external. The sensory system consists of sense organs located mostly at the free surfaces of the body, both internal and external, which convey information to the central nervous system. The endocrine organs influence such matters as rate of growth, size attained, rate of chemical changes in the body, sexual development, and similar general processes.

The integumentary, muscular, and skeletal systems have already been noted. The other systems will now be described briefly.

1. Circulatory system.—There are two circulatory systems in vertebrates: the *circulatory system proper* or *blood-vascular system* and the *lymphatic system.* The latter has already been noted as consisting of large spaces filled with *lymph.* The lymph circulates slowly and eventually empties into the blood-vascular system. The blood-vascular system is composed of definite tubes, the *blood vessels,* in which a red fluid, the *blood,* flows rather rapidly in a continuous circuit. The parts of this system are the *heart,* which by its beating causes the flow, the *arteries* (vessels in which the blood flows away from the heart), the *veins* (vessels in which it flows toward the heart), and the *capillaries* (microscopic vessels between the ends of the arteries and the beginnings of the veins). Cut off the pericardial sac with a fine scissors and examine the heart. It consists of several parts called the *chambers of the heart,* which in the frog are as follows: *ventricle, auricles* (two), *sinus venosus,* and *conus arteriosus.* The ventricle is the thick-walled, cone-shaped part, pale in color; its posteriorly directed point is named the *apex* of the ventricle, its anterior end the *base* of the ventricle. Anterior to the base of the ventricle are two thin-walled sacs, right and left, dark red in fresh specimens,

the *right* and *left auricles*. Extending from the right side of the base of the ventricle obliquely forward between the two auricles is a tube, the *conus arteriosus* (often but erroneously designated in books as the bulbus arteriosus). The tubes leading away from the conus are the main *arteries*. To locate the sinus venosus, lift the ventricle (cutting through a small ligament by which the ventricle is attached) and bend the heart forward so that the apex points anteriorly. Identify a small chamber appearing as a posterior continuation of the auricles from which it is separated by a transverse white line. This chamber is the *sinus venosus*. It receives from the liver and from around the sides of the auricles, large vessels, dark red in fresh specimens, the principal *veins*. Through these veins the blood returns to the heart and is pumped out again through the arteries.

2. Respiratory system.—This consists of the external nares, nasal cavities, internal nares, mouth and pharyngeal cavities, glottis, larynx, and two lungs. Most of these parts have already been seen. The *larynx* ("Adam's apple" of man) is a chamber situated inside the laryngeal prominence and will not be studied at this time. If the lungs were not previously identified locate them as follows. Push other organs to one side and look in the dorsal part of the coelome to the sides of the heart for a dark-colored cone-shaped organ attached at its broad end to the anterior wall of the coelome on each side. These organs are the lungs. A windpipe (*trachea*) is lacking in the frog.

3. Digestive system.—This may be considered as continuous tube, the *alimentary canal*, extending from mouth opening to anus, with several other structures attached to it. Mouth opening, teeth, mouth cavity, and pharynx were already noted. The *esophagus* is a short stout tube which leads away from the pharynx but cannot be seen at this time. It passes into the stomach, already noted, from which a narrow coiled tube, the *intestine*, extends to the anus. Note attachment of stomach and intestine to the mid-dorsal wall of the coelome by a mesentery, the *dorsal mesentery*. In this mesentery near the posterior end of the intestine is situated a small round red body, the *spleen*. Associated with the alimentary canal are two digestive glands, the *liver*, already noted, and the *pancreas*. The latter is an indistinct yellowish material (more distinct in the bullfrog) spread out irregularly in the mesentery which extends between the liver, intestine, and stomach.

4. Reproductive system.—This consists in each sex of a pair of *reproductive glands* or *gonads* in which the sexual elements are formed, and a pair of *ducts* which convey these elements to the exterior.

The gonads of a female are known as *ovaries* and their ducts as *oviducts*. These have already been noted in mature specimens. In immature specimens, the ovaries are small ruffled objects, white with black specks, fastened to the dorsal wall of the coelome; and the oviducts are small white tubes lateral to the ovaries. The black and white objects in the ovaries are the female sex elements, which are called *eggs* or *ova*.

The gonads of a male are known as *testes* and their ducts as *vasa deferentia* (singular, *vas deferens*). In the frog the testes are two small oval yellow bodies fastened to the dorsal wall at about the middle of the trunk. The testes in the frog and other lower vertebrates have no ducts of their own but use the ducts of the kidneys. In the leopard frog and some other species (but not in the bullfrog), *rudimentary* oviducts are present

in the male; they are fine white tubes one lateral to each testis and attached directly to the dorsal wall.

Attached to the anterior end of each gonad is seen a tuft of yellow finger-shaped processes, called the *fat body*. This structure is a storehouse of fat which is used by the frog in the winter when the animal is inactive and does not feed. The fat body is therefore large in the fall and small in the spring.

5. Excretory system.—This consists in vertebrates of a pair of *kidneys* and their *ducts*. To find the kidneys of the frog turn all of the viscera to the right and study the dorsal wall of the coelome. Note that here the peritoneum is not fastened to the muscular layer but that there is a large cavity between the peritoneum and the muscles. This space is called the *subvertebral lymph sinus* or *cisterna magna*, and is, like the subcutaneous lymph spaces, a part of the extensive lymphatic system which the frog possesses. In the cisterna magna certain structures, including the kidneys, are located. Such structures are said to be *retroperitoneal*, i.e. they lie outside the peritoneum. On the left side break through the peritoneum which forms the ventral wall of the cisterna magna. Located in the cisterna the *kidneys*, a pair of elongated flat dark-red or brown bodies situated close to the peritoneum, which passes along their ventral faces. From the lateral posterior edge of each kidney arises its duct, which is named the *mesonephric duct* (also called *wolffian duct* and *vas deferens* and, incorrectly, *ureter*). At the posterior end of the coelome is the *urinary bladder*, previously identified. It functions as a reservoir for the secretion of the kidneys, a fluid called *urine*.

The mesonephric ducts convey not only the urine but also the male reproductive elements to the exterior. Owing to the existence of such close connections between the excretory and reproductive systems in many animals these two systems are commonly treated together as the *urogenital system*.

It may be mentioned that the kidneys of the frog although having the same function as the human kidneys do not exactly correspond to the latter from a morphological point of view. For very accurate purposes the kind of kidney which the frog possesses is designated a *mesonephros* or *mid-kidney* while that of man is a *metanephros* or *hind-kidney*. The meaning of this statement can be understood only after a study of the embryology and comparative anatomy of vertebrates.

6. Endocrine system.—Under this head are gathered together a number of glands or glandlike bodies which secrete into the blood substances of great importance to the life and health of the animal. The pancreas and reproductive organs belong to this system but have, of course, other important functions as well. The *adrenal gland*, one of the endocrine organs, forms a bright yellow stripe on the ventral face of each kidney. The *pseudothyroids* are a pair of small reddish bodies on the throat just in front of the heart. The *thyroids* are situated just internal to the pseudothyroids but the student should not attempt to find them. For the location of other glands of internal secretion and the functions of these glands textbooks of physiology should be consulted.

7. Nervous system.—The nervous system is divisible into three parts: the *central nervous system*, consisting of the *brain* and the *spinal cord*; the *peripheral nervous system*, consisting of the *nerves* which pass between all parts of the body and the brain (*cranial* nerves) and the spinal cord (*spinal* nerves); and the *sympathetic nervous sys-*

tem, a special part of the central nervous system which affects the involuntary activities of the body (as heart beat, movements of the digestive tract, etc.).

Turn the frog back upward and remove the muscles from the head and mid-dorsal region of the back. This exposes the skull in the head and a row of spiny projections, the *neural spines* of the vertebrae, along the median line of the back. With a sharp scalpel cut horizontally through both external nares and continue posteriorly cutting or, better, peeling away the bones of the middle part of the skull. The white lobed *brain* is thus revealed lying in a cavity, the *cranial cavity,* of the skull. Continue posteriorly cutting off the dorsal parts of the vertebrae. A white cord, the *spinal cord,* will be seen to lie in a cavity of the vertebrae, called the *neural canal.*

The cranial nerves cannot be observed at present. The spinal nerves arise in pairs from the spinal cord between successive vertebrae. Turn the frog ventral side up and look into the cisterna magna. The stout white cords which appear here closely applied to the muscles of the dorsal body wall are the most posterior spinal nerves.

The sympathetic nervous system consists mainly of a nervous strand on either side of the mid-dorsal line of the coelome. At regular intervals along these strands there occur enlargements or *ganglia* which are connected by nerves with the adjacent spinal nerves. Owing to their small size and delicate nature, the sympathetic strands are a little difficult to find. Have the assistant help you locate them in the roof of the cisterna magna, alongside a large artery (*dorsal aorta*).

8. Sense organs.—The principal sense organs, the *olfactory sacs* (nasal cavities), *eyes,* and *ears* have already been noted. In addition to the *middle* ear which was seen, there is another part of the ear, the *internal* ear, buried in the skull and impractical to dissect. There are *sense organs* in the skin which serve to detect light, contact, chemicals, differences in temperature, injury, etc., and *taste* organs in the lining membrane of the mouth cavity.

Make a diagram of a cross-section through the posterior part of the trunk of the frog. Show the layers of the body wall, the subcutaneous lymph spaces, the positions of the skeletal parts present at this level, the cisterna magna and contained kidneys and the coelome. In the coelome draw a circle to represent a section of the intestine and draw the relation of the peritoneum to this section of the intestine, showing mesentery and visceral peritoneum. This drawing is to be constructed from the knowledge gained in this chapter.

The general anatomical features and relations expressed in such a diagram are common to all vertebrates and in fact to all animals which possess a coelome.

D. GENERAL ANATOMICAL PRINCIPLES

Now that we have made a survey of the anatomy of the frog, attention may be called to some general principles which underlie its construction.

1. Principle of bilateral symmetry.—The parts of the frog are arranged symmetrically with reference to the median sagittal plane, being either *paired* or *unpaired.* Among the unpaired structures are the skull and vertebral column, brain and spinal cord, heart, digestive tract, and chief blood vessels. All of the muscles, the appendicular parts, the nerves and chief sense organs, lungs, kidneys, reproductive organs and their

ducts, and many of the blood vessels are paired. The digestive tract is the chief unsymmetrical system in the body but it originally was a straight median tube.

2. Principle of cephalization.—At one end of the frog is a structure termed the head which is distinguished by its extensive content of nervous tissue. The head bears the main part of the nervous system, the brain, and the chief sense organs. *Cephalization* is the name applied to the production of a head by the accumulation of nervous structures in one part of the body. For such accumulation a long process of evolution is necessary.

3. Principle of segmentation.—Less obvious than the two foregoing features is the *segmental arrangement* of the parts of the frog. By segmental arrangement is meant that the parts of the body are repeated at regular intervals along the antero-posterior axis. In a perfectly segmented animal, the body would be constructed of a longitudinal row of sections or *segments* each one of which would bear a paired or unpaired representative of each part of the body as a pair of limbs, pair of kidneys, pair of gonads, etc. In the adult frog remnants of such a segmented condition are seen in the vertebral column, consisting of a longitudinal row of vertebrae, in the spinal nerves of which there is a pair to each vertebra, and in many of the muscles and blood vessels.

E. ECOLOGY OF THE FROG

We have hitherto studied the anatomical parts of the frog much as one would study the anatomical parts of a machine. But just as the parts of a machine all work together to accomplish some one purpose, so all of the parts of the frog act in correlation with each other, maintaining the frog as a living thing.

Each animal in nature lives in a certain kind of place which is called its *habitat*. Each animal in its habitat is surrounded by and subject to a number of conditions which together are spoken of as its *environment*. Among the factors which go to make up an animal's environment may be mentioned: *chemical* conditions, such as composition of water, soil, etc.; *physical* conditions, as light, temperature, water content of the air, rate of evaporation; *physiographic* and *meteorological* factors, as climatic and seasonal changes, topography of the habitat, kinds of rocks and soils present, etc.; and *biological* factors, that is, the plants and other animals living in the same habitat. Each animal in order to survive must be adjusted to the conditions of its environment throughout the year. The frog as we all know lives in and around ponds and streams. Its structure and its behavior are fitted for this kind of habitat and it cannot survive if placed in a habitat considerably different from its natural one.

The study of the characteristics of environments, the kinds of animals living in each environment, and the adjustments of animals both in structure and behavior to their environments constitutes a branch of zoölogy called *ecology*. That part of ecology which deals with the reactions and behavior of animals is frequently designated as *animal behavior*. It attempts to find out the responses of animals to the factors of their environment and the causes of these responses. Experiments in this field of biology have shown that animals tend to move toward or away from or are indifferent to the factors of the environment. Thus some animals go toward light and avoid darkness, others avoid light and so move into dark places, while others are indifferent to the presence or ab-

sence of light. A response of an animal to an environmental factor is called a *tropism* or *taxis* and these words are combined with a prefix to indicate the factor concerned, as *phototropism* or *phototaxis,* reaction to light. The tropism or taxis is said to be *positive* if the animal moves toward the factor in question, *negative* if it avoids it.

The following simple observations and experiments are presented to illustrate to the student something about the behavior of an animal and the correlation between its structure and its behavior. *Write down full notes while watching the animal* and later write up in your notebook a full account of what you saw.

Note sitting posture of the frog. How are the fore limbs held? hind limbs? Observe sacral hump. Make the frog jump and note exact way in which the legs are used in jumping and in landing. Can the frog jump from the sitting posture without delay? Is this of advantage?

Place a frog in a tank of water and note swimming movements, especially use of the webs and hind limbs. Are the fore limbs used in swimming? Compare swimming movements of the frog with the "breast stroke" used by man. How long does the frog stay under water? In water too deep for the frog to sit on the bottom with head out, note the floating attitude. Why does the frog have to keep its head out of water? Place in the tank an object tall enough to project out of the water. By what behavior does the frog find the object? Do you think the frog "realizes" there is something in the water on which it can sit? Does it go directly toward the object as we would? Cause a frog sitting out of the water to dive into the water. Note use of the legs in diving and compare with human method of diving. Can the frog dive from the sitting posture without delay? Is this of advantage? Do you think the hind limbs of the frog are better constructed for jumping, swimming, and diving than our legs are?

In a frog sitting quietly note respiratory movements. These consist of a rapid rise and fall of the floor of the mouth cavity, less rapid opening and closure of the external nares, and expansions and contractions of the lateral walls of the trunk. These movements will be studied again later.

Determine by making appropriate movements whether the frog sees and whether it feels a touch. Can you suggest an experiment to determine whether the frog can hear or not? If so, try it on the frog.

Has the frog a sense of *equilibrium,* that is, of its position? Determine this by the following experiments. Turn the frog over on his back. Note righting movements. Place a frog in a covered dish and after it has become quiet tilt the dish in various ways. What is the behavior of the frog? Slowly rotate the dish in a horizontal plane. Observe what the frog does. Rotate the dish in the opposite direction and again note response. Can you explain this behavior of the frog on the assumption that the animal attempts to keep its eyes fixed on one object? Would this behavior be of use to the frog in attempting to catch an insect? The ability of the frog to recognize changes of position is due to certain parts of the internal ear. Read in textbooks of physiology about the semicircular canals and their functions.

Observe the feeding behavior of the frog. The frog feeds on insects, earthworms, and in fact any small animals. Place a frog in a covered dish with a little water and add some flies or other insects or an earthworm. How long before the frog perceives the

presence of food? From its behavior do you think the frog sees, hears, or smells the food? Observe the attitude assumed by the frog with reference to the food and action of the tongue in capturing the food. Put a dead fly into the dish. Does the frog show any response to a dead (i.e., non-moving) object of food? (Frogs will generally not feed in the winter time.)

Determine the reactions of the frog to some of the most important factors of its environment. For example, if a number of frogs in a tank or aquarium are available note whether they take up any position with regard to the lights and shadows of the tank or with regard to the direction of the rays of light. Or put some frogs in a large pan with a little water, darken half or three-fourths of the pan and after some time note position of the frogs. Are frogs positive or negative to light? A tendency to move toward or into lighted places is called *positive phototaxis*, an avoidance of such places *negative phototaxis*. Do frogs tend to aggregate, i.e., collect in groups or to place themselves under stones or other objects? A tendency to remain in contact with other animals or other objects is called *positive thigmotaxis*. Can you suggest any advantage in such a reaction? Determine if frogs respond to moisture. Arrange a large pan with a layer on the bottom of material that will hold water as sand, earth, sphagnum, etc. Wet one half of the bottom thoroughly and leave the other half dry. Have all other factors such as light equal throughout the pan. Place some frogs in the pan, cover with a screen, and from time to time note where the animals are found. When an animal moves toward or into moist places or water, such a reaction is called *positive hydrotaxis;* the opposite reaction is called *negative hydrotaxis*. Is this reaction of importance to the frog? Can the frog live if its skin becomes dry?

Note that the skin of the frog is cold and slimy to the touch. The frog is a cold-blooded animal, that is to say, its temperature is approximately the same as that of its surroundings. It cannot keep its body warm in cold weather as we do but as winter comes on the animal becomes colder and colder and finally passes into a state of inactivity known as *hibernation*. Why cannot a cold-blooded animal continue to be active at a cold temperature? The sliminess of the frog's skin is due to the mucus secreted by glands of the skin. Do you think this mucus slows down the rate of evaporation of water from the surface of the skin? Can you suggest any other advantages of a slimy skin?

As a result of the foregoing study the student should realize that there is a remarkable correspondence between the behavior of the frog, the structures which it possesses, and the characteristics of its environment. This correspondence between environment, habits, and structure is called *adaptation*. It is not known by what processes animals become adapted; in fact, this is one of the most important biological problems. Neither can we answer such questions as to whether the frog took up the habit of jumping because it had legs constructed for jumping or whether it developed legs fitted for jumping by trying to jump. Adaptation is a universal characteristic of living things and the power of becoming adapted seems to be one of the properties of living matter.

II. GENERAL PHYSIOLOGY: PROPERTIES OF LIVING MATTER

The preceding chapter considered chiefly the structure or *morphology* of an animal, with some observations on its behavior as a whole. The present one will be devoted to a study of the uses or *functions* of the *parts* of an animal, a branch of biology called *physiology*. To determine the functions of parts we must necessarily study them *alive* for function is inseparable from life and disappears with death. The physiological phenomena described herein are common to animals in general.

A. IRRITABILITY, CONDUCTIVITY: FUNCTIONS OF THE NERVOUS SYSTEM

Irritability is perhaps the most striking property of living matter. It may be defined as the capacity of living matter to undergo a change as a consequence of changes external or internal to itself. The change in the living thing is known as the *reaction* or *response* and in the case of animals the response generally becomes visible as a *movement*. The change which produces the response is called the *stimulus;* the act of applying a stimulus to living matter is called *stimulation*. The response may appear at a different point from that to which the stimulus was applied, in which case it is evident that the stimulus was *conducted* within the living matter. This ability of living matter to transmit stimuli is known as *conductivity*. The time which elapses between the application of the stimulus and the appearance of the response is called the *reaction time*.

Irritability and conductivity are properties of all living matter. In a complex animal like the frog they are particularly *specialized* in the *nervous system;* in fact to perceive, conduct, and respond to stimuli is the function of the nervous system. The following experiments are designed to prove this.

An experiment has been defined as a question put to nature. To answer a question about nature we arrange a certain set of conditions and watch what happens. "What happens" is nature's answer to our question under the particular set of conditions which we arranged. Obviously if these conditions are not properly arranged the answer that we get will not be the correct answer to our question, or several answers may be possible. Setting up the right combinations of circumstances is the first and most important step in an experiment. Next we must observe very carefully and accurately what happens. If we miss part of the response the answer we get is evidently only partly correct. Finally we must think about what we saw, attempt to reason logically from the results, and so reach conclusions.

In the following experiments *watch carefully* what happens and *write this down as fully and accurately as you can at the time that you do the experiment. Do not depend upon memory.*

Obtain from the assistant a frog pithed in the brain only. Suspend it by a wire through the tip of the lower jaw from any convenient object. Wait until it hangs quietly. Have a pan or dish of tap water handy to keep the frog moist and wash off the acid in the following experiment. Do not allow the frog to become dry. Dip a very small

piece of filter paper about $\frac{1}{16}$ inch square, into dilute acetic acid and stick it to the skin of the abdomen of the frog. What happens? This is the response. Does the place to which the stimulus (the acid) was applied itself respond? Does this prove that conduction occurred? Does the experiment tell us by what part or system of the frog conduction occurred? Wash off the piece of paper with water and repeat, determining with a watch the approximate reaction time; put the acid paper on other parts of the body, as the toes, hind legs, back, chest, etc. Wash off the frog thoroughly after each trial. Which part of the body is the most sensitive as determined by the shortness of the reaction time? Is the response different according to where the acid is placed? When are the fore limbs used? Does the reaction appear to be purposive, i.e., to be performed with a definite useful end in view? Remember that the brain of the frog was destroyed.

Does the experiment so far prove that the nervous system has anything to do with the reaction? Have the assistant pith the spinal cord of the frog. Hang it up again and repeat the experiment putting the acid paper on the skin of the abdomen as before. Is there any response? Have we now proved that the central nervous system is necessary for the response? What part of the central nervous system?

The reaction of the frog when acid is placed on the skin is called a *reflex action* or more briefly, a *reflex*. This particular reflex is named "the wiping reflex." The term reflex implies that a circuit is involved and this is the case. The parts of this circuit have been determined by the experiments of physiologists and are as follows: stimulation of the sense organs in the skin by the acid; conduction of this stimulation along the nerves leading from the skin to the spinal cord; conduction in the spinal cord to the level where the nerves to the muscles of the limbs originate; an impulse from this level of the spinal cord along the nerves to the particular muscles needed; contraction of these muscles producing appropriate movements of the limbs. Just what parts of this circuit did our experiment prove to be involved in the wiping reflex? What parts did we not prove to be involved?

Write up this and all subsequent experiments in your notebook according to the following suggestions. As the reports of students on experiments have generally proven to be *unsatisfactory*, the method of writing up the present experiment is given in some detail.

1. *Purpose of the experiment:* State the object of the experiment. This means explain what the experiment is intended to show or prove. The object of the experiment under consideration is to demonstrate that living matter is irritable (responds to stimuli) and conductile and that the nervous system is involved in these properties.

2. *Conditions of the experiment:* As these are stated in the directions, it is not necessary for the student to repeat them in his report. He should have them clearly in mind, however, as the meaning or significance of the result depends upon the conditions under which the experiment is carried out.

3. *Result:* State fully and accurately what happened in the experiment. Answer the questions asked in the manual about the experiment. The value of this part of your report will depend on the fulness of the notes which you took at the time of performing the experiment. State what the frog did when the acid was applied at various places,

whether the result was different according to place stimulated and what the difference was, the reaction time for different places stimulated, etc.

4. *Conclusions:* Draw conclusions from the results which you stated above. Understand that drawing a conclusion is a mental process, an exercise in reasoning. Your ability to draw conclusions from what you saw in the experiment is for your instructor a measure of your mental keenness. The process of drawing a conclusion may be illustrated from the present experiment as an example to the student. The acid is applied to the skin, say of the abdomen. This is one fact. The place to which the acid is applied does nothing, makes no visible response. This is a second fact. The hind legs move in certain ways. This is a third fact. We draw the conclusion that the effect of the acid on the skin was somehow conducted through the animal to the legs. Observe that the experiment itself does not give us this conclusion directly. It gives us only certain facts. The conclusion is a logical process in our own minds. Note further that from the three facts stated we cannot draw any conclusion as to how the stimulus of the acid was conducted or what structure of the frog conveyed it. We can, however, set up experiments that will give us additional facts from which we can draw conclusions about the path followed in the conduction of the stimulus. The last part of the experiment gave us some of these facts, but not all of the necessary ones. The student should realize that all statements in books about the functions of organs or parts depend upon experiments similar to those given here and represent conclusions drawn from such experiments.

State as your own in your report only those conclusions which are justified by the facts observed by you in your experiment. Think clearly about just what the experiment proved and what it did not prove. For instance, note that although the experiment tells us something about the function of the nervous system it does not prove that this system might not have other functions as well.

At the end of your report on the experiment additional facts and conclusions found in your reading may be stated but make it clear that you obtained these statements from books or elsewhere and did not prove them in your experiment.

B. CONTRACTILITY: FUNCTION OF THE MUSCULAR SYSTEM

Contractility is the ability of living matter to effect movement by changing its form. This property is particularly evident in muscles.

1. Contractility of voluntary muscle (muscle under the control of the will).— Remove the hook from the jaws of the frog used in the preceding section. Lay the frog back upward, make a circular incision through the skin around the base of the thigh, and grasping the cut edge of the skin, completely strip the skin from the hind leg. On the dorsal surface of the thigh, three muscles will be seen: laterally, the *gluteus magnus;* medially, the *semimembranosus;* and between these, the small slender *ilio-fibularis.* On the inner surface of the shank is a large spindle-shaped muscle, the *gastrocnemius.* Carefully separate the ilio-fibularis from the semimembranosus and locate between them the *sciatic nerve,* appearing as a stout white cord, running alongside a dark-colored blood vessel. Carefully separate the nerve from the blood vessel being very careful not to injure either. Lift up the nerve, keep your eye on the gastrocnemius

muscle, and cut through the nerve. What happens? What is the stimulus? How does the stimulus get to the muscle? How does the reaction time compare with that in the preceding experiment? Can you explain why? The experiment may be repeated as many times as desired by again cutting the nerve between the first cut and the muscle. Satisfy yourself that in the motion the muscle actually becomes shorter and thicker. How does such a change of shape cause movement of the leg? Understand this by raising your forearm by contraction of the biceps muscle.

2. **Contractility of involuntary muscle** (muscle not under control of the will, found mainly in the walls of the viscera).—Turn the frog ventral side up and cut through the ventral body wall to the left of the median line from the pelvic girdle up through the pectoral girdle. Gently pull the stomach out so that it will be clearly exposed and with a forceps pinch the wall of the stomach. Wait for the response. What happens in the stomach wall after a little time has elapsed? How does the reaction time compare with that of voluntary muscle? Watch the contraction travel. In which direction does it go? This kind of contraction is called peristalsis. What is its purpose (*R*)?

3. **Contractility of heart muscle.**—Remove the pericardial sac from the heart and study the beating of the heart. The beating of the heart is a rhythmical contraction of the muscular walls of its chambers. Observe changes in color, form, and size of the ventricles, auricles, and conus arteriosus with each beat. In what order to these three parts beat? Turn the heart with the apex forward so that the sinus venosus can be seen. Does the sinus beat? What time relation does its beat bear to that of the other parts of the heart?

Count and write down the number of heart beats per minute. Then cool the heart by placing small pieces of ice around it and after it has become thoroughly chilled again count the rate of the heart beats per minute. This experiment shows the effect of temperature upon the rate of activities of living matter. What is the effect?

Remove the heart by cutting through the posterior end of the sinus venosus and anterior end of conus arteriosus. Place it on a clean glass plate in a little salt solution. Does the heart continue to beat when removed from the body? We saw above that the contraction of a voluntary muscle is normally caused by the stimulation of its nerve. Is the heart beat caused by nerves going into it, judging from this experiment? If the heart has stopped beating, pinch it with a forceps. Result?

4. **Ciliary motion.**—Open the mouth of the frog, swab it out with water and if necessary cut the angle of the jaws to keep it open. Lay the frog ventral side up and sprinkle a little powdered carmine or place small bits of cork on the posterior part of the roof of the mouth. Observe that the particles travel as if carried on a current. (If the experiment fails it is because the frog has been pithed too long, and a fresh frog may be necessary.) The current is due to a multitude of microscopic projections, the *cilia,* which cover the roof of the mouth, somewhat like bristles on a brush, and by their co-ordinated beating produce a current of mucus sufficiently strong to carry fairly large particles. Prove that the particles will be transported against the force of gravity by repeating the experiment with the head of the frog tilted at an angle, so that the particles must be carried uphill. In which direction does the ciliary current run and what

is its purpose? While the causes of ciliary motion are not at all understood, it is probable that this kind of motion is also a form of contractility.

C. DIGESTION, ABSORPTION: FUNCTION OF THE DIGESTIVE SYSTEM

The food of animals in general consists of water, salts, and organic substances. An organic substance is one produced by a living creature, either plant or animal. The organic substances which serve as foods are divided into three main classes, *proteins, carbohydrates,* and *fats,* each of which is distinguished by definite chemical characters. The proteins are the only ones of the three classes of organic foods which contain nitrogen. They are spoken of as nitrogenous foods and are the chief source of nitrogen for the body. Examples of proteins are meat, white of egg, curdle of milk, cheese, blood clot, gelatin. Sugars and starches, the white parts of cereals, potatoes, white rice are examples of foods that are wholly or mostly carbohydrates. Butter, cream, the fat parts of meat, lard, and olive oil, and fats and oils in general used for cooking are examples of fats.

While the water and salts pass into the substance of the animal without alteration, the carbohydrates, fats, and proteins are too complex to be absorbed and used by the animal and must be broken down into simpler substances before they can be utilized. This splitting of the organic foods into simpler utilizable substances is the *process of digestion* and the performance of this process is the function of the alimentary tract and its glands. The digestion of foods, that is, the splitting of complex foods into simpler substances, is brought about by means of certain substances, called *enzymes,* which are manufactured in the walls of the alimentary tract and in the digestive glands, chiefly the pancreas. Enzymes are so far as known at present produced only by living creatures. They are remarkable substances which have the power of speeding up chemical reactions which would occur very slowly without them or which can be made to occur rapidly only by the use of boiling temperatures and reagents which would be fatal to life. Neither the chemical nature nor the mode of action of enzymes is known; but it is suspected that they are proteins and that they act by attaching themselves to the molecules of the substance to be digested, thus upsetting the equilibrium within the molecule, and causing it to fall into fragments, whereupon the enzyme is liberated unchanged. Although enzymes can be made only by living creatures and are found only in living things, they are not themselves alive. They can be extracted from animals and plants and their digestive action on foods studied in the laboratory.

There are a number of different kinds of enzymes found in plants and animals. A remarkable property of enzymes is that each one acts on only one substance or class of substances. There are three general types of enzymes for each of the three general classes of organic foods. The enzymes which split up proteins are known as *proteases;* those which split carbohydrates as *diastases;* and those which split fats as *lipases.*

The chemical changes which each kind of food undergoes when an enzyme acts on it are well known but cannot be understood without considerable knowledge of chemistry. A brief statement may help the student understand what follows. Each food goes through a definite series of chemical changes, or stages of digestion, which are the more numerous the more complex the food was at the beginning. Proteins pass through the

following stages: proteoses, peptones, peptids, and amino acids, which last are the end products of protein digestion. Starches and other complex carbohydrates are split into dextrins (several stages of dextrins) and complex sugars, and these finally into simple sugars, chiefly glucose. Glucose is the main end-product of carbohydrate digestion. Fats are broken up into glycerin and fatty acids.

In the following experiments, an attempt is made to show the student something of the action of each of the three principal classes of enzymes. We cannot here, of course, study the action of enzymes thoroughly as this would require a great deal of chemical knowledge and training. As it is rather impractical to obtain sufficient enzymes from frogs for class work owing to their small size, human and pig enzymes having the same action are used instead.

1. Action of a protease—pepsin.—

a) *The gastric juice:* This digestive fluid is secreted by glands located in the wall of the stomach. It contains about 0.4 per cent of hydrochloric acid, a protein-digesting enzyme called *pepsin,* and small quantities of a number of other substances. An "artificial" gastric juice is readily made by adding 0.4 per cent hydrochloric acid to commercial dried pepsin, generally obtained from the hog's stomach.

b) *Action of pepsin:* Into a test tube put 5–10 cc. of artificial gastric juice and into another 5–10 cc. of 0.4 per cent hydrochloric acid. Add to each a small quantity of the white from a hard-boiled egg, cutting the white into very small pieces. Place in a water bath or incubator kept at 37°C. (why?) for at least two hours. What happens to the protein? Is the acid alone without the pepsin capable of producing this effect? Pepsin from the frog's stomach has the same action.

2. Action of a lipase—pancreatic lipase.—

a) *Reaction of milk:* To 10 cc. of fresh milk (or better, cream) in a test tube add a few drops of neutral litmus solution or test with red and blue litmus paper. Litmus is a vegetable dye which turns pink in acid solution, blue in alkaline solution, and purplish in neutral solution. Judging from the color resulting when the litmus is added to the milk, is milk acid, alkaline, or neutral?

b) *Action of pancreatic lipase:* A solution of this enzyme is obtained by dissolving dried pancreas, sold commercially as pancreatin, in a slightly alkaline solution. To the litmus-containing milk prepared in (*a*), add half its quantity of the pancreatin solution. What color is the mixture? From the color, decide whether the mixture is acid, alkaline, or neutral. Into another test tube put 10 cc. of milk containing litmus, but do not add any pancreatin solution to this. Place both test tubes, one containing only the milk and litmus, the other containing milk, pancreatin solution, and litmus, into a water bath kept at 37°C. Observe from time to time. Does the color of the litmus change? In both tubes? In which one? What does this change indicate? This action of the pancreatic lipase is of course upon the cream (fat) of the milk.

3. Action of a diastase—ptyalin.—

a) *Test for starch:* Place a few cc. of a 1 per cent starch paste in a test tube and add a drop or two of iodine. A deep blue color results, which is due to a chemical union between the iodine and the starch. This reaction is a *test for starch.* This means that no other substance but starch gives a blue color with iodine and that, therefore, if any-

thing turns blue when we add iodine to it, we know that starch is present in it. (Try the test on clothing that has been starched.)

b) *Test for simple sugar:* Place a few cc. of glucose (fruit sugar) solution in a test tube, add a few drops of Fehling's solution, and heat to boiling. A yellowish, greenish, or red precipitate is formed. This is a test for sugar, that is to say, when we add Fehling's solution to an unknown solution and get the reaction, this proves that sugar (certain kinds of sugar) is present. Does Fehling's solution by itself contain any sugar? Boil some of it to see. Result?

c) *Test the starch paste* with Fehling's solution. Does the starch paste contain any sugar?

d) *Spit some saliva* from your mouth into a test tube. Test it with Fehling's solution. Does it contain any sugar? (If you have been eating candy or anything sweet, wash the mouth out thoroughly before using your saliva for this test.)

e) *Action of saliva:* Prepare three test tubes as follows. Into one put nothing but 5 cc. of starch paste. Into another put nothing but some of your saliva. Into a third put 5 cc. of starch paste, and spit some saliva from your mouth into the starch paste; mix the saliva thoroughly with the starch paste. Put all three tubes into the water bath at 37°C. for several minutes. Remove and test each test tube with Fehling's solution. Is sugar present in any of them? Which? Where did it come from? Is the presence of saliva necessary for its production? The experiment gives us all of the necessary facts for concluding where the sugar came from and you should be able to decide this without resort to textbooks. The enzyme in the saliva which has the action observed is called ptyalin (from a Greek word meaning "spit"). An enzyme having a similar action is secreted by the pancreas of the frog (the mouth secretions of the frog have no digestive effect).

Write up these experiments in your notebook according to the plan already suggested. Give the purpose of each experiment. State exactly what happened in each experiment and answer the questions asked in the manual. Draw conclusions from what happened, stating what each of the enzymes did to each particular food, *in so far as tested in the experiment, and in so far as the experiment showed.* As before, avoid presenting conclusions which are not justified by what you saw in the experiment. Thus for instance, the experiment on saliva tells us that sugar was produced and what it came from but does not tell us the various chemical stages in the process. After writing up your own observations and conclusions, material obtained from textbooks may be added, but state clearly that such material is taken from books and does not originate from your experiments.

4. Absorption.—After the food is digested by the aid of enzymes, the products of digestion, which are simple soluble substances, pass through the wall of the intestine. This process is called *absorption* and is very little understood. The end products of the digestion of proteins and carbohydrates pass into the blood, of the digestion of fats into the lymph. This is proved by testing chemically the blood and lymph which flow away from the intestine. It is not practical for elementary students to make such tests or observe the process of absorption.

5. Egestion or defecation.—A certain part of the food eaten by animals is indigestible. This means that they do not possess any enzymes capable of breaking down such materials into utilizable substances. This indigestible material is known as *feces* and is voided to the outside through the anus. The act of discharging the feces is known as *egestion* or *defecation*. It should not be confused with excretion discussed a little later.

D. RESPIRATION, OXIDATION: FUNCTION OF THE RESPIRATORY SYSTEM

When any organic material burns it uses up a gas, *oxygen*, found in the air, and gives off another gas, *carbon dioxide*. In exactly the same way living animals burn either their own substance or the materials obtained from digestion, using up oxygen and releasing carbon dioxide. All of the processes involved in this burning are called collectively *respiration*. The purpose of the respiration of living matter is the same as our burning of wood or coal, namely, to produce *heat* and set free *energy*. If we burn a piece of wood we can produce heat to warm ourselves or we can make steam, for instance, by means of which we can do work such as running a locomotive. Similarly, in our bodies we burn the food we eat or our own substance to keep our bodies at a certain temperature or to yield energy for our various activities. Animals and plants are able to carry on this burning process, or respiration, without becoming very hot, by the aid of enzymes. We can burn sugar with the aid of a flame; our bodies burn sugar at ordinary temperature (98°F. in the case of man) by the aid of enzymes.

1. External respiration in the frog.—In very small animals and in plants oxygen is taken in and carbon dioxide discharged directly through the surface of the body. But in larger animals, some mechanism is necessary for getting the oxygen into the body and throwing off the carbon dioxide. This mechanism constitutes *external respiration*. It is commonly called "breathing."

Obtain a live frog, place it in a covered dish with a small amount of water, and after it has become quiet study the *respiratory* (breathing) *movements*. These consist of the opening and closure of the external nares, the rise and fall of the floor of the mouth cavity, and the contraction and expansion of the sides of the trunk. Time the rate of each of these movements. Do any of these three movements take place *simultaneously?* Are the nostrils open during the movements of the floor of the buccal cavity? Would these movements cause air to pass in and out of the nostrils? What happens to the nostrils when the sides of the body expand? Does the frog breathe in the same manner as man? Man takes air into his lungs by increasing the size of the chest cavity. This is done by raising the ribs through the contraction of muscles attached to the ribs and by lowering the diaphragm through contraction. The chest is thus made larger and our nostrils being open all of the time air rushes into the lungs. Now as we learned, the frog has no diaphragm, neither has it any ribs. Can it, therefore, breathe as we do? The frog can get air into its lungs only by swallowing the air. Could it swallow air if the nostrils were open? What must, therefore, happen to the nostrils when air is to be forced into the lungs? Recall that the upper and lower jaws of the frog fit together very tightly. Do you now understand why? Could the frog get air into its lungs if its mouth were open or were not air-tight? Read further on the respiration of the frog

in Holmes' *Biology of the Frog*. Understand thoroughly the way in which a frog breathes and write an account of it in your notebook including your own observations.

2. Respiratory activity of the skin.—Demonstration experiment. Four jars, each containing a frog, are placed in a trough of running water, so that the temperature of all is the same. One jar is filled with running water, the second with standing water, so that all bubbles of air are entirely excluded, the third has only an inch or two of water in the bottom, and the fourth is quite dry. Do the frogs completely immersed under water show any respiratory movements? How do they get oxygen? What is the condition of all of the frogs after two or three days? Must the skin of the frog be kept wet, if the animal is to continue to live? Can you give reasons for this? State the results of this experiment in your notebook and explain the results as fully as you can.

3. Carbon dioxide output.—The following simple experiment demonstrates that carbon dioxide is given off from the lungs. (This need not be performed by those familiar with it.) This test depends upon the fact that carbon dioxide forms a white insoluble compound with lime water or similar solutions. A bottle is provided containing two or three inches of lime water (or saturated solution of barium hydroxide), and provided with two tubes one of which extends below the lime water, while the other does not. First suck on the shorter tube, thus drawing air from the room through the lime water. Does anything happen? Next blow air from the lungs into the longer tube. Does anything happen? Does this experiment prove that the air which comes from our lungs is different from that which we breathe in? The carbon dioxide from our lungs forms the white compound seen in the lime water when we blow into it.

To prove that the frog also gives off carbon dioxide, place a frog in a dish with a little water in the bottom. Inside the dish set another smaller dish containing lime water. Close larger dish tightly with a glass cover. Observe if the lime water becomes cloudy.

4. Internal respiration or oxidation. The respiratory movements and mechanisms considered in 1 and 2 are merely methods of getting oxygen into the body and into the blood and carbon dioxide out. The actual use of the oxygen by the living substance is the real process of respiration. To avoid confusion this process is designated as *internal respiration* or *oxidation*.

E. EXCRETION: FUNCTION OF THE EXCRETORY SYSTEM

Every part of the animal in carrying on its activities use up or burns (oxidizes) its own substance and food materials. Just as ashes remain when fuel is burned, so certain materials, called *waste products,* are left over from the activities of the body. These waste materials are not only useless to the body but they are even poisonous and must be eliminated in a relatively short time or the animal cannot live. The waste materials from the oxidation of carbohydrates and fats are chiefly in the form of gases (carbon dioxide and water vapor) and are easily cast off from the body in the expired air from the lungs and (in the frog and many other animals) through the skin. But in the utilization of proteins by the body, some of the wastes left over are not in the form of gases and cannot be gotten rid of in such a simple fashion. These waste products of proteins contain nitrogen and are called *nitrogenous wastes*. Special organs are neces-

sary to eliminate these nitrogenous wastes from the body. Such organs constitute the excretory system. As we have already learned, the kidneys, their ducts, and the urinary bladder constitute the excretory system of the frog. The kidneys do not themselves produce the nitrogenous wastes, except the small amount resulting from their own activities. The kidneys collect these wastes from the blood. Every part of the body gives off wastes into the lymph and from the lymph into the blood and as the blood circulates through the kidneys they extract the nitrogenous and all other non-gaseous wastes from the blood and cenentrate then in a little water, forming a fluid called the *urine*. It is not known how the kidneys do this. The urine is passed down the ducts of the kidneys and in the frog may be ejected directly or held for a time in the bladder.

Excretion may be defined as the elimination from the body of the waste products, gaseous and non-gaseous, of body activities. Although the lungs (and in some animals, the skin) take part in the excretion of gaseous wastes, the term excretory system is generally confined to organs which are engaged in the active concentration of nitrogenous and other non-gaseous wastes. It is preferable that the term excretion should not be applied to the process of defecation.

The proof of the excretory function of the kidneys is too difficult to be undertaken by beginning students.

F. CIRCULATION OF THE BLOOD AND LYMPH: FUNCTION OF THE CIRCULATORY SYSTEM

The food prepared in the digestive tract and the oxygen taken in through the lungs (and skin) must be carried to all parts of the body or they will soon die; and the waste materials must be rapidly removed. The blood-vascular system serves for the rapid transport of these materials; the lymph acts as an intermediary fluid between the blood vessels and the living matter of the body.

1. Circulation of the lymph.—The lymph circulates mainly through body movements, but in the frog (not in man) there are two pairs of *lymph hearts* which aid the flow by their rhythmical contractions. Remove the skin from the back of a recently pithed frog. Look to each side of the posterior tip of the urostyle for a small colorless sac, beating rhythmically. The other pair of lymph hearts is less easy to see. They are between the transverse processes of the third and fourth vertebrae.

2. Circulation of the blood.—In the web of the frog's hind foot spread out under the microscope, observe the following:

a) The network of tubes, the *blood vessels*, in which the blood is contained.

b) The composition of the blood. It consists of solid bodies, the *corpuscles*, which are seen shooting along the vessels, and a colorless fluid, the *plasma*, in which the corpuscles are suspended.

c) *The arteries*, vessels in which the direction of flow is from the larger vessels into their smaller branches.

d) *The veins*, vessels in which the direction of flow is from the smaller vessels into the larger.

e) *The capillaries*, the smallest vessels, forming a network, in which the direction of flow is indefinite.

f) *The pulse,* rhythmic jerks in the flow, due to the heart beats, present only in the arteries, observable by watching the corpuscles, which jump at each heart beat and move less rapidly between beats.

The student will probably notice the black spots in the skin of the webs. These are color bodies or *chromatophores.* They are capable of movement and thus exhibit various shapes; when contracted they are round black masses; when expanded they show long, delicate, spidery processes. The frog has two or three other kinds of color bodies besides the black chromatophores.

G. REPRODUCTION, DEVELOPMENT, THE LIFE-CYCLE: FUNCTION OF THE REPRODUCTIVE SYSTEM

The ovaries produce the female elements, which are called *eggs* or *ova,* and the testes produce the male elements, called *spermatozoa* or, more briefly, *sperm.* The egg cannot develop in most animals unless it unites with a single spermatozoon, a process known as *fertilization.* The *fertilized* egg then undergoes a process of *development* which results eventually in the production of an individual like those from which the egg and sperm came. The complete history from one individual to the next is called the *ontogeny* or *life-cycle.*

Not very many animals are suitable for the study of the life-cycle in the laboratory owing to the length of time required in most cases and the limitation of the breeding season to certain times of the year. In the spring the development of the frog can be studied in the living condition if material is available. The development of the frog is described in chapter vi. Under most circumstances insects are more suitable and we shall here describe the development of a fly. The small fruit fly (Drosophila) is readily reared under laboratory conditions. In the spring and summer the blow-fly can be employed. In the former case each student or table will be supplied with a bottle containing banana mash and a pair of fruit flies. If the blowfly is to be used, put an inch or two of moist sand in the bottom of a wide-mouthed bottle. Place a piece of fresh liver on the sand and set near an open window. Watch the gathering of blowflies about the bottle, and observe, if possible, the laying of eggs upon the liver by the female flies. Allow flies to escape and then stopper the bottle with a wad of cotton.

1. The flies.—Obtain a Drosophila or other fly killed by ether and note its characteristic *insect* structure: the division of the body into *head, thorax,* and *abdomen;* the characteristic rings or *segments* composing the abdomen; the large *compound eyes;* the three pairs of *legs;* and the single pair of *wings* (most insects have two pairs of wings but flies have only one pair). Learn to distinguish males from females. In Drosophila, the males have slender rounded abdomens with a black area on the tip of the ventral side; females have broader more pointed abdomens, marked with black at the sides of the ventral surface. In blowflies, the large eyes nearly meet in front in males, while in females they are considerably separated.

2. The eggs.—Record date on which eggs are first observed. Note where they are laid. They are oval white objects, very minute in the case of Drosophila. Examine one under the microscope. Its surface is marked with hexagonal sculpturings which are the impression of the walls of the oviduct; the Drosophila egg is provided with two

oarlike projections which prevent it from sinking into the soft banana pulp. **Draw** an egg.

3. The larvae.—A process of development goes on inside the eggshell, resulting in the formation of a *larva* or *maggot,* which hatches by rupture of the shell. The larvae are white, wormlike creatures. Record date on which they are first seen. Examine an etherized larva with low power. Compare its structure with that of the parent fly. Is it more simple? Does it have head, eyes, wings, legs? Is the body divided into regions? Is the ringing (segmentation) of the body more marked than in the parents? This extreme difference between the larval and the adult structures is believed to represent an adaptation to a mode of life very different from that of the parents. For a discussion of adaptation see page 15. The original mode of development of insects was probably very different from that of the fly, and this modification of development through adaptive alteration is called *caenogenesis.*

Striking structures present in the larva are the *tracheal tubes,* a pair of longitudinal tubes which branch extensively throughout the body. They open to the exterior by a large posterior and a smaller anterior pair of openings, the *spiracles.* The tracheal tubes are filled with air which they distribute throughout the body, constituting in fact the respiratory system of the animal. This respiratory system of air tubes is peculiar to insects and their relatives; in other animals oxygen is carried by the blood. The anterior protrusible end of the larva is a very imperfect *head,* with a mouth leading into a pharynx provided with hard black curiously shaped *jaws.* Each segment of the larva is encircled with a girdle of small spines, used to prevent slipping. The interior of the body is filled mostly by the coils of the *digestive tract* and with yellowish masses, the *fat bodies,* in which excess food is stored. A sac, the *food reservoir,* an outgrowth of the esophagus, may be noticeable if filled with food. Two slender brown or black tubes, intermingled with the coils of the digestive tract, are the excretory tubules or *Malpighian tubules,* characteristic of insects. **Draw** a larva on a large scale.

Note behavior of living larvae, method of crawling, use of the girdles of spines, use of the jaws in taking food. Where do the larvae stay? Which side of the meat do the blowfly larvae frequent? What use do they make of the sand?

Note remarkably rapid increase in size, that is, *growth* of the larvae. Observe that this is not gradual but occurs by jumps, a method peculiar to insects and their relatives. The outer layer of these animals is inelastic and they can increase in size only by shedding this layer (molting). Whenever they molt a sudden increase in size is noted. Consider that in growth the dead food is transformed into living substance, a process called *assimilation.*

4. The pupae.—After attaining a certain size the larvae cease to feed and transform into motionless objects, the *pupae,* inclosed in brown *pupal cases,* formed from the outer layer of the larvae. Record date when pupae are first seen. Where are they located? Examine a pupa under the microscope. Is it segmented? Has it any head? Are the girdles of spines and spiracular projections present? **Draw** a pupa. Inside the pupal case, the larva disintegrates and the adult fly develops from certain of its rudiments. As time passes note adult structures appearing in the pupa, seen through the pupal case.

5. **The adults** (imagines).—Record the date on which adult flies are first seen. Note that the pupal case is ruptured and left behind by the fly. Is the place of rupture the same for all individuals? How many days were required for the entire life-cycle? Would the time be the same for all seasons of the year? Why? How many flies were produced? Proportions of males and females? If all of the offspring lived to reproduce at the same rate, how many flies would there be at the end of three months? What factors in nature prevent this enormous increase in numbers? From this study of the development of the fly, do you think that flies grow? Are small flies the young of larger flies?

The life-cycle of the fly is typical of one kind of life-cycle found among animals, in which the form which hatches from the egg is very different in appearance from the parent and attains the parent condition by sudden changes, called *metamorphoses,* as the change from larva to pupa and pupa to adult. The transformation of the tadpole or polliwog into the frog is another example of metamorphosis. In these cases the creature hatching from the egg is called a *larva.* Many animals, however, do not pass through any *larval stages* but on hatching greatly resemble the parent and attain the adult condition by growth.

H. SUMMARY OF PHYSIOLOGICAL PROCESSES

1. The food prepared in the digestive tract by the action of enzymes is absorbed through the walls of the intestine into the circulatory system.

2. Oxygen drawn into the lungs by the mechanical arrangement of the respiratory system (or taken through the skin in some animals) passes through the walls of the lungs or through the skin into the circulatory system.

3. The circulatory system transports food and oxygen to all parts.

4. The living substance of the body withdraws the food and oxygen from the blood by way of the lymph and uses them:

a) For the formation of new chemical compounds or of new living substance (process of assimilation) thus accomplishing growth.

b) Or for the release of energy by burning the food material (or its own substance) with the aid of the oxygen (process of oxidation or true respiration).

5. The waste products resulting from the oxidation or other chemical processes are given off into the blood which carries them to the lungs, skin, and kidneys, where they are thrown out from the body. The lungs give off mainly carbon dioxide and water; the skin, water, minute quantities of dissolved waste matters, and in some animals carbon dioxide; the kidneys excrete nitrogenous and other non-gaseous waste matters, dissolved in water.

6. The combined processes of assimilation, oxidation (or other energy-liberating changes) and excretion are spoken of together as *metabolism.* Metabolism may be defined as a sum of those chemical changes taking place in living matter which result in the production of new compounds, new living matter, or the release of energy.

7. The energy released in the metabolic processes is utilized to carry on body activities such as contraction of muscles, conduction of nerve impulses, secretion of digestive fluids, etc.

III. GENERAL HISTOLOGY: CELLS AND TISSUES

The material of which living bodies are composed is named *protoplasm*. Protoplasm does not occur as large masses but is divided up into or exists as minute portions each of which is called a *cell*. A cell is defined as a small mass of protoplasm containing a characteristic body, the *nucleus*. All living things are composed of a number of cells or consist of one cell. The study of the kinds of cells of which animals and plants are composed is called *histology*.

As cells are of minute size the microscope is necessary for their study. Since only thin transparent objects can be viewed in the microscope, plants and animals and their parts must be cut into thin slices called *sections* and these must be so treated as to render them transparent, if they are to be studied histologically. In making sections the piece of animal or plant desired is killed by chemicals that preserve it as nearly as possible in its natural condition, and after being hardened by chemicals is imbedded in a block, usually of paraffin. The paraffin block containing the material is set in a machine called a microtome. In the microtome the block is moved up and down across a very sharp razor edge which slices off very thin sections of the material in paraffin. Such sections may be cut as thin as $\frac{1}{1000}$ of a millimeter but are usually $\frac{1}{200}$ to $\frac{1}{100}$ of a millimeter thick. The sections are mounted on slides, the paraffin dissolved away, the material stained (in order to render its parts more visible) by dyes similar to those used in dyeing cloth, treated with chemicals that remove water and render the material transparent, and finally covered with a sticky material called balsam and a cover glass.

The student will now understand that owing to the numerous processes and chemical agents to which the material is subjected in making sections, artificial and abnormal appearances are frequently produced. Further a difference in the angle at which the material is sliced will make two sections of the same material present a different appearance. In studying sections the student should always bear these points in mind, and *should direct his attention only to those portions of the sections pronounced by the instructor to be typical and normal and illustrative of the points under consideration.*

Read carefully and follow the general directions at the beginning of this book on the use of the microscope and consult the instructors on any points which you do not understand.

A. STRUCTURE OF A TYPICAL CELL

Sections of glands constitute favorable objects for the study of cell structure. Certain Amphibia, such as Necturus, relatives of the frog, are commonly used as material, because their cells are of unusually large size.

Examine under low power a section through the liver of Necturus. Note that the liver is composed of polygonal blocks, like a mosaic. Each of these blocks is a *cell* and we thus see that the liver is composed of such cells. A study of sections through all parts of this or most other animals and plants would show that they are similarly constructed.

Examine a liver cell under the high power. The principal parts of a cell are: the *cell membrane*, the delicate partition which bounds each cell, the *nucleus*, the central dark body in each cell, and the *cytoplasm* or remaining material of the cell.

1. The cell membrane.—This probably consists of two parts, the actual surface of the protoplasm, called the *plasma-membrane*, and a material secreted by the cell, often designated as the *cell wall*. It is probably the latter that ones sees in these liver cells.

2. The nucleus.—This is the oval deeply stained body in the center of the cell. It is inclosed in a membrane, the *nuclear membrane*. The nucleus consists of two parts, the visible solid material, having the form of a network, and the invisible material, called *nuclear sap* or *enchylema*, which fills the spaces of the network. The network itself is composed of at least two parts, a framework, the *linin* or *achromatic* network, and the conspicuous deeply staining granules or masses, the *chromatin*, which are strung on the fibers of the linin network and partly conceal it from view.

3. The cytoplasm.—The *cytoplasm* or *cytosome* is that portion of the protoplasm outside of the nucleus. It consists of solid visible materials imbedded in an invisible material, which is called the *ground substance* or *hyaloplasm*. The solid structures of the cytoplasm are very variable in different cells and their visibility depends almost entirely upon the chemicals and dyes used in preparing these cells for microscopic study. Fibrils, networks, granules of various shapes, sizes, and chemical composition, and droplets of fat or other fluids are among the regular constituents of cytoplasm. In the cytoplasm of the liver cell of Necturus, the solid material appears as a network, called the *spongioplasm*. It is doubtful if this represents the real structure of the cytoplasm; probably it is the result of the coagulating action of the chemical used to kill the cells.

Draw a liver cell under high power, showing all details.

B. CELLS AND TISSUES

The liver cell studied above is an example of a *typical cell*, that is, one having the usual form and structure characteristic of cells in general. In a complex animal, however, there are many types of cells, some of which are highly *specialized*, that is, that are modified in form and structure for the performance of particular functions. We find further, in examining the microscopic structure of an animal, that although some cells occur free and isolated from others, the majority of them are united into layers or groups composed of like cells. Cells may also secrete materials, known as *intercellular substances*, which remain associated with them. A group of like cells together with the intercellular substances (if any) secreted by them is called a *tissue*. There are four principal kinds of tissues and hence of cells in animals—*epithelial*, *muscular*, *connective*, and *nervous*. We shall now study examples of the various kinds of tissues and cells found in an animal like the frog.

NOTE.—In these studies of tissues, it is *absolutely essential* that the student observe the following directions: (1) All material must be mounted in liquid, either water (if dead) or physiological salt solution (if living). (2) All material must be spread out as thin as possible or picked to minute pieces with a pair of teasing needles. It is useless and a waste of time to try to study thick material. (3) After having made the material

as fine as possible put on a cover glass, lowering it from one edge to avoid air bubbles. Absorb excess fluid with filter paper; the cover glass should not float around on the material. (4) Study the tissue first with low, then high power, without staining, and draw what you can see in the unstained tissue. (5) Then stain, if so directed (for method of staining see general directions), and add to the drawing already made any additional structures revealed by staining. Avoid using excess stain. (6) If what you see in your preparation does not correspond with the description in the manual, you are probably looking at the wrong thing; consult the instructor. (7) Draw only a few cells, making your drawings large and detailed, putting in every structure that you can see.

1. Epithelial cells and tissues.—Epithelial cells resemble in appearance the "typical" cell already studied. They always occur in sheets, such a sheet constituting an *epithelial tissue* or, as it is more generally called, an *epithelium*. An epithelium is composed of one or more layers of epithelial cells with very little intercellular substance between the cells. Epithelia clothe the external surface of the body and line all internal surfaces and cavities. *Glands* are formed by infoldings of epithelia. There are several kinds of epithelial cells and epithelia of which only a few are here considered.

a) *Squamous epithelium:* This kind of epithelium is composed of very flat cells. Obtain a small piece of shed epidermis (outer layer of the skin) of the frog, spread it out on a slide in a drop of water, cover, and examine with low power. Cut down the light. The epidermis is seen to be composed of flat polygonal cells, arranged like a mosaic. Study a cell with high power noting its nucleus and (in some cases) the dark pigment granules in the cytoplasm. If the nucleus is not evident, stain with a drop of aceto-carmine. By changing the focus of the microscope determine whether the epidermis is one or more layers of cells thick. Draw, showing a few of the cells.

b) *Columnar epithelial cells:* This type of epithelial cell is tall and slender. Obtain some of these cells from the inner lining of the intestine which has been soaked in chloral hydrate to loosen its cells. Note their elongated shape, broader at one end, narrower and frequently irregularly branched at the other. In some of the cells the broader end contains a cup-shaped cavity filled in life with mucus; such cells are called *goblet cells*. The oval nucleus (if it is not visible apply the aceto-carmine stain) is usually nearer the narrow end and causes a slight bulging of the cell. Draw a few of the cells.

c) *Ciliated epithelium:* In this type of epithelium, the free surface bears cilia. Obtain from the assistant a little tissue scraped from the roof of the mouth of a recently pithed frog. Add a drop of salt solution, tease into small bits, cover, and examine. Search the field until a shimmering movement is seen; then examine this with high power. Note groups of cells or single cells with one surface covered with delicate hair-like processes of the cytoplasm called *cilia*, which keep up a rapid vibration, sufficiently strong to move small particles in the vicinity or to cause the cells themselves to whirl around. Note shape of the cells, a shape called *cuboidal;* isolated cells tend to become rounded. Observe the appearance of *living protoplasm* as seen in the cytoplasm of these cells. The nucleus may be rendered visible by applying the aceto-carmine stain but this will kill the cells. Draw. Recall experiment 4, under B, chapter ii, where the function of these cells was seen.

2. Muscle cells.—Muscle cells are characterized by a long and slender shape, fibrillar structure, and power of contractility. They form no intercellular substance but are aggregated into layers or masses with the aid of connective tissue.

a) Smooth (involuntary, unstriated) muscle cells: Obtain a small piece of intestine that has been soaked in chloral hydrate, tease into small bits, cover, and examine. Observe the very long, slender, almost threadlike *smooth muscle cells,* running parallel in layers. They are best seen on the edges of the material. Each cell possesses an elliptical nucleus; stain if necessary. **Draw.**

b) Striated (voluntary) muscle cells: Cut out a small elongated piece from the leg muscles of a preserved or recently pithed frog (the former is often better), tease carefully lengthwise until you have separated the piece into long threads, cover and examine. The long cylindrical objects you will see are the *muscle cells,* or *muscle fibers;* they are so long that one usually fails to obtain a complete one, the ends being, therefore, broken. Each voluntary muscle is made up of a great many such muscle fibers (cells) bound together by connective tissue. Each fiber is inclosed in a delicate cell membrane, the *sarcolemma,* and is composed of alternate light and dark disks, which being viewed on edge, appear as cross stripes, hence the name "striated" applied to these cells. A longitudinal striation may also usually be seen, indicating that the muscle fiber is really composed of a number of much smaller longitudinal fibers, the *fibrillae* or *sarcostyles,* bound together in the same sarcolemma. Each muscle fiber contains a number of slender nuclei situated just under the sarcolemma; stain to see them. **Draw.**

3. Connective tissue.—In this type of tissue the cells are few in number and scattered and there is a large amount of intercellular substance. Connective tissue is widely spread throughout the body, being used to bind and support other parts and tissues. There are several kinds of connective tissue of which only a few are here mentioned.

a) White fibrous or collagenous connective tissue: This is the white weblike material binding the muscles of the frog together, forming the partitions between the lymph sacs, and in fact of wide occurrence. Remove a little from a preserved frog, spread it out carefully on a slide to a thin layer, add water, cover, and examine. It consists mainly of long, slender, wavy fibers, the *white* or *collagenous* fibers. Another type of fiber, the *yellow elastic,* which runs straight and singly, is present in small numbers. Connective tissue fibers are *intercellular* products and are probably not living. The connective tissue cells are few in number and are scattered among the fibers. To see them, apply the aceto-carmine stain and after a few minutes note the stained oval nuclei of the connective tissue cells. The acetic acid in the stain also tends to dissolve the white fibers making the yellow elastic ones more distinct. Cells and fibers are imbedded in an invisible gelatinous *matrix,* also an intercellular product. **Draw a small portion of the tissue.**

b) Cartilage: Examine a thin slice of living cartilage from a frog, mounting it in salt solution, or use the slide of cartilage provided in your set. Observe that cartilage consists of a clear firm material, the *matrix,* in which there are present at intervals rounded cavities, the *lacunae,* which are completely filled in life by the *cartilage cells.* These cells secrete the matrix. Often two cells will be seen in one lacuna; they have

originated by the division of one cell and will become separated by the secretion of matrix between them. **Draw** a small portion of cartilage.

c) *Bone:* Examine prepared slide of a section of bone. As such slides are prepared from dead bone, the bone cells and other living structures are absent. The bone consists of a matrix rendered very hard and strong by the deposition in it of mineral salts, mostly calcium phosphate, through the activity of the bone cells. The matrix is arranged in concentric layers, called *lamellae,* around circular holes, which are the cross-sections of canals, the *Haversian canals.* These canals traverse the bone in a longitudinal direction and in life carry blood vessels, etc., to the bone. Scattered through the lamellae are minute spaces, the *lacunae,* from which spidery processes, the *canaliculi,* extend into the lamellae. In life the lacunae are occupied by the *bone cells,* whose processes extend out into the canaliculi. **Draw** a portion of bone.

4. Blood.—Blood is difficult to classify but is sometimes regarded as a type of connective tissue in which the intercellular substance is fluid. The cells of blood are called *corpuscles,* the intercellular fluid part, the *plasma.* There are two general kinds of corpuscles, the *red corpuscles* which give the red color to the blood and carry oxygen, and the *white corpuscles* which protect the body from disease and destroy foreign objects.

a) *Fresh blood:* Obtain from the assistant a drop of fresh frog blood, stir it up in a little salt solution, cover and examine with high power. The numerous oval bodies are the red blood corpuscles. Note that they are not red when viewed singly. By gently moving the cover glass, cause the red corpuscles to float about, and as they turn determine their shape in profile. The central bulge is due to the nucleus, which is generally faintly visible. Look for white corpuscles, scattered here and there among the red corpuscles, small, irregular in shape and with granular cytoplasm. If found, watch for some time and you may see them undergo slow changes of shape, an example of *amoeboid movement.*

b) *Stained blood:* Examine a slide of blood with the high power. Note:

(1) The *red blood corpuscles,* the numerous oval objects on the slide, each with a central nucleus. **Draw.**

(2) The *white blood corpuscles* or *leucocytes.* As these are much less numerous than the red corpuscles it will be necessary to search the slide carefully for them. There are several kinds of leucocytes, classified into three general groups: *lymphocytes, granulocytes,* and *thrombocytes.* Try to find some of the following and make drawings of those seen:

a) *Lymphocytes:* These are rounded cells of various sizes, each containing a large central spherical nucleus encircled by a narrow rim of cytoplasm.

b) *Granulocytes:* These possess a granular cytoplasm and usually a complex nucleus, consisting of two to several pieces, united with each other by delicate strands. There are three kinds of granulocytes: *cosinophil,* with coarse red granules in the cytoplasm; *basiphil,* with coarse blue or purple granules; and *neutrophil,* the most common kind, with fine pale granules. The first and last are *polymorphonuclear,* i.e., they have the complex nucleus just mentioned. The basiphil granulocytes have an ordinary spherical nucleus.

c) *Thrombocytes:* These, also called *spindle cells,* are small cells of irregular form, usually spindle-shaped, with a single spherical nucleus, and a small quantity of granular cytoplasm.

5. Nervous tissue.—Nervous tissue is composed of *nerve cells* and the structures which support them. A nerve cell consists of a central nucleated portion, the *cell body,* from which springs numerous *processes.* These processes are of two kinds: *dendrites,* which convey impulses into the cell, and are usually numerous and much branched; and *axones* which convey impulses away, and are always single and generally unbranched. Portions of nervous tissue composed of cell bodies are known as *gray matter;* portions composed of the processes, as *white matter. Nerves* consist of bundles of *axones.*

a) *Brain cells:* Thick sections of the brain of mammals furnish good examples of brain cells. As the processes of nerve cells do not usually show up very well when stained by ordinary methods, a method devised by an Italian histologist named Golgi, in which the cells are blackened by silver, is frequently employed. Examine sections of mammalian brain stained by Golgi's method. The numerous black branched objects on it are nerve cells. Study one of these (*A*). In sections through a part of the brain called the cerebrum, each brain cell has a more or less triangular form and bears a large stout process from its pointed end, and several branched processes from its broad end. Another part of the brain called the cerebellum has a different type of brain cell, with a round cell body, two or three highly branched tree-like *dendrites* from one surface, and a small slender *axone* from the opposite surface. **Draw** a brain cell.

b) *Motor cells of the spinal cord of the frog:* Examine the cross-section through the spinal cord of the frog under low power. The central large oval body is the section of the spinal cord (all other structures are to be disregarded). In this section observe a central, denser, slightly darker region, the *gray matter,* trapezoidal in shape, and containing numerous darkly stained cell bodies; and an outer region, lighter, more open, with only a few cell bodies, the *white matter.* In the two corners of the lower base of the trapezoid formed by the gray matter locate several very large cells. These are the *motor cells* whose axones extend out to the voluntary muscles. Examine one of these under high power. The structure of the cell body is well shown, but only the beginnings of the processes are present. (Why?) Study the *structure* of the *cell body,* comparing it with other cells seen. Note the relatively large *nucleus,* containing a conspicuous round body, the *nucleolus,* but very few chromatin granules, and the granular, sometimes fibrillar structure of the cytoplasm of the cell body and bases of the processes. **Draw** one motor cell under high power showing details.

c) *Motor cells of the spinal cord of cattle:* These slides are made by mashing a small bit of the spinal cord after staining in carmine. Note several large branched cells among the material on the slide. These are the *motor cells* of the spinal cord, that is, the cells of origin of the nerve fibers that go to the voluntary muscles. Examine one of these cells with the high power. Note central part of the cell, called the *cell body.* It contains a nucleus inside of which is a large spherical body, the *nucleolus.* From the cell body spring several *processes.* The cytoplasm of the cell body and of the processes is more or less distinctly fibrillar. **Draw** one of the cells.

d) *Structure of a nerve:* Obtain a small piece of fresh nerve from a recently pithed frog. Mount it in salt solution and tease with needles in a longitudinal direction until you have separated it into minute fibers. Cover and examine. The nerve is seen to be made up of a number of cylindrical fibers, bound together by connective tissue. Each of these fibers is a single process (*axone*) of a nerve cell. Find an isolated fiber and examine with the high power. It consists of a central transparent region, the axone itself, often called here the *axis cylinder,* and a thick sheath surrounding this, which is known as the *medullary sheath.* This sheath is composed of a fatty substance called *myelin,* which tends to swell up and become distorted. Outside of the medullary sheath is a delicate cell membrane, the *neurilemma.* **Draw.**

6. **Reproductive cells.**—Reproductive cells are, when mature, free cells and cannot, therefore, be classified under the head of tissues. They are, however, derived from epithelial cells.

a) *Egg cells or ova:* The female reproductive cells known as *eggs* or *ova,* are among the largest of all cells, the yolk of the hen's egg, for example, being one cell. The eggs of the frog are the large black and white spherical bodies seen in the ovaries. The structure of ova may be studied in sections through the frog's ovary or in whole eggs of such animals as the starfish or sea-urchin. Note spherical form, definite membrane, highly granular cytoplasm, and large vesicular nucleus, with a large amount of nuclear sap, very little chromatin, and a conspicuous nucleolus. **Draw.**

b) *Spermatozoa:* The male reproductive cells or *spermatozoa* are highly specialized modified cells, bearing little resemblance to most cells. Those of the frog are best studied in the spring as at other seasons they are apt to be imperfect. Mammals furnish suitable material at all times. Take a small bit of the testis of a frog or rat or guinea-pig, etc., or of the seminal vesicle of an earthworm, tease in salt solution, cover, and examine. Thousands of minute, slender, rapidly moving *spermatozoa* will be seen. Each consists of a *head,* usually rounded or oval, rodlike and curved in the frog, which is practically nothing but the nucleus, and a long, very slender, vibratile *tail,* which represents the cytoplasm. **Draw** a spermatozoon.

IV. GENERAL HISTOLOGY: STRUCTURE OF ORGANS

An *organ* consists of two or more kinds of tissues united together in a definite and characteristic way for the performance of specific functions.

A. STRUCTURE OF THE LIVER

The liver may be taken as an example of a rather simply constructed organ and also as a *secreting gland*. Like all multicellular glands it consists almost entirely of epithelial cells with a small admixture of other tissues. Examine a section through the liver of Necturus or the frog. Note the large polyhedral epithelial cells arranged in cylindrical *columns* which branch and fuse in a very irregular manner, forming a kind of network with numerous large spaces between the columns. These spaces are capillaries of a peculiar kind, known as *sinusoids*, serving to bring the blood in very close contact with the liver cells. Note the blood corpuscles in the sinusoids. A very slender, usually invisible, canal, the *bile capillary*, runs down the center of each liver column and in life receives a fluid, the *bile*, secreted by the liver cells. The large black spots scattered through the liver are collections of *pigment granules*. Large blood vessels and perhaps some of the *bile ducts* which collect the bile from the bile capillaries may be present on the section. Surrounding the liver and penetrating it here and there along the courses of blood vessels and bile ducts is a small amount of white fibrous connective tissue.

B. STRUCTURE OF THE SKIN

The skin is a combination of epithelial and connective tissue with a small amount of smooth muscular tissue. The epithelial part is external and is called the *epidermis;* the connective tissue part is internal and is named the *dermis* or *corium*. Examine a section through the skin of the frog.

1. **The epidermis.**—The epidermis or outer layer of the skin is a *stratified epithelium, i.e.,* an epithelium composed of several layers of cells. The outermost layers or *stratum corneum* consist of squamous epithelial cells, flattened and horny, already studied in the preceding chapter. The stratum corneum is frequently shed by the frog, and is then replaced by the following layer. Internal to the stratum corneum, the cells gradually change from a flattened to a rounded, and finally to a columnar, shape, these layers constituting the *stratum germinativum* (also called *stratum Malpighii*). The cells of this stratum may contain brown pigment granules and dark-brown or black branched pigment cells (*chromatophores*) may be scattered among them.

2. The dermis.—The dermis is composed of connective tissue, separable into two layers, an outer loose layer, the *stratum spongiosum,* which contains large round bodies, the *cutaneous* or *skin glands,* and an inner compact layer, the *stratum compactum*.

a) *Stratum spongiosum:* This consists of a loose irregular network of connective tissue fibers, inclosing blood vessels, lymph spaces, etc. It contains a thin layer of *chromatophores,* situated just beneath the epidermis.

b) *Cutaneous glands:* These are hollow flask-shaped bodies, composed of a single-layered epithelium, produced by simple infoldings of the stratum germinativum of the epidermis. Each gland opens to the surface by means of a narrow *neck,* which passes through the epidermis. Explain why most of the glands appear to have no neck and why some of them seem to be solid instead of hollow. The skin glands of the frog are of two general kinds, *mucus* and *poisonous.*

The mucus glands are smaller and much more numerous than the poison glands and occur on all sections of the frog's skin. Their appearance varies with their degree of activity. When inactive, their epithelial cells are tall and columnar and the cavity of the gland reduced; when active, the cells are cuboidal in shape and the enlarged cavity of the gland is filled with masses of mucus. This mucus serves to keep the skin slimy.

The poison glands are of large size and infrequent occurrence, being most abundant in the dermal plicae. They will not be present in all sections. They consist of a thin epithelial wall surrounding a very large cavity in which the secretion appears as granular masses near the epithelium. The secretion is not poisonous to man but appears to be so to small animals.

c) *Stratum compactum:* This consists of a dense layer of white fibrous connective tissue, running parallel to the surface of the skin. At intervals vertical strands, consisting of connective tissue, smooth muscle cells, blood vessels, nerves, etc., cross the wavy layers of the stratum compactum at right angles and may extend up into the epidermis.

d) *Subcutaneous connective tissue:* Beneath the stratum compactum occurs loose connective tissue which binds the skin to the underlying muscles.

Draw a portion of the section, showing in detail the structure of the skin.

C. STRUCTURE OF THE INTESTINE

The wall of the intestine consists of epithelial, connective tissue, and smooth muscle layers, arranged in a definite fashion. Examine a section through the intestine of Necturus or the frog. The sections should preferably have been stained with Mallory's triple stain, a stain which dyes connective tissue and related substances blue, thus producing a strong contrast between the various layers of the intestinal wall. The following description applies, as to colors stated, to slides stained in this way; if other stains are used, the colors will be otherwise.

1. **General structure of the intestinal wall.**—The intestinal wall consists of four layers. Identify these as follows under low power, beginning next the cavity.

a) *The mucous coat* (*mucous membrane* or *tunica mucosa*), the innermost light-colored layer, thrown into folds.

b) *The submucous coat* (*tela submucosa*), a broad band, stained blue, extending into the folds of the mucosa.

c) *The muscular coat* (*tunica muscularis*), remaining light-colored layers of the intestinal wall, consisting of two strata: the *circular* muscle layer, in which the muscle fibers run in a circular direction, and the *longitudinal* muscle layer, in which they run lengthwise.

d) *The serous coat* (*tunica serosa,* or *visceral peritoneum*), a very thin membrane covering the outside of the intestine, invisible under low power.

2. Detailed structure of the intestinal wall.—Select a favorable place where the mucous membrane is cut parallel to the long axes of its cells. (*A*). Study under high power.

a) The mucous membrane consists of the *lining epithelium* and subjacent parts. The lining epithelium is composed of a single layer of tall columnar epithelial cells, arranged with their long axes at right angles to the cavity. These cells were studied in chapter iii, B. Each is bounded by a cell membrane, often indistinct, and possesses near its base a large oval granular nucleus, stained red or orange. Why do the nuclei appear so numerous (*A*)? Are they all in the same plane (determine this by changing the focus of the microscope)? The free ends (ends next the cavity) of many of the epithelial cells contain flask-shaped depressions or *goblets*, filled with mucus, which stains blue with Mallory's stain as mucus is chemically similar to connective tissue. There are thus two kinds of cells in the intestinal epithelium, the ordinary *columnar cells*, and the *goblet cells*. Understand the different appearance of various parts of the mucosa by considering that the plane of the section may not necessarily be parallel to the long axes of the epithelial cells.

Immediately external to and running parallel to the epithelium is a layer of loose white fibrous connective tissue, designated the *tunica propria* or *stroma*, which is considered to be part of the mucosa. It contains numerous connective tissue cells (of which only the nuclei are distinctly visible) and is abundantly perforated with lymph spaces and blood vessels.

b) The submucous coat is composed of white fibrous connective tissue continuous with the tunica propria, which it resembles in structure.

c) The circular muscle layer consists of smooth muscle cells running parallel to the plane of the section. The boundaries of the individual muscle cells are not clear but the whole layer appears to consist of parallel fibrils. The conspicuous nuclei are long and spindle-shaped.

d) The longitudinal muscle layer is composed of smooth muscle cells running at right angles to the plane of the section and thus appearing as circles, which are imbedded in white fibrous connective tissue, stained blue. Understand why the nuclei may or may not appear in the cross-sections of the muscle cells. Numerous blood vessels are present in this layer.

e) The serous coat is a very thin membrane looking like a line applied to the outer surface of the preceding coat. At intervals its flattened nuclei are seen. The serous coat is not really part of the intestinal wall but is the visceral peritoneum, which clothes all of the organs in the pleuroperitoneal cavity.

Draw a small portion of the intestinal wall showing its structure accurately and in great detail. This drawing should be four or five inches wide.

D. STRUCTURE OF THE STOMACH

Examine a section through the stomach of the frog. Observe that the general appearance and arrangement of layers of the stomach wall are as in the small intestine, except that certain coats are thicker (which ones?). The particular point of interest about the stomach is the presence of *glands* in the mucosa.

1. The gastric glands.—The mucosa of the stomach is thrown up into folds or *rugae* which appear in section as conical elevations. The lining epithelium does not cover the rugae evenly but is itself folded in and out producing a large number of long *tubular glands* set closely together with their long axes parallel and at right angles to the surface. It may be noted in passing that all glands are formed by such infoldings of epithelia. Narrow strands of tunica propria separate the glands from each other. Find a place on the slide where the glands are cut parallel to their long axes and examine a gland under high power. Its appearance differs somewhat in different parts of the stomach. The principal parts of a gastric gland are the *mouth* or *duct, neck,* and *body* or *fundus*. The duct is the longest part and contains a narrow canal leading from the interior of the gland to the cavity of the stomach. The cells at the opening into the stomach are very elongated with clear free ends, probably containing mucus, and elongated nuclei. Farther down the duct, the cells and nuclei become more rounded. The region at the junction of duct and body consists of a few clear cells and is called the neck. Here the gland may branch, two or more bodies being attached to one duct. The body or fundus of the gland is composed of polygonal granular cells, whose function is the secretion of the gastic juice.

Draw a gastric gland in detail.

2. The muscularis mucosae.—The stomach also differs from the intestine in that the tunica propria of the mucous membrane is separated from the submucosa by a narrow band of smooth muscle fibers, called the *muscularis mucosae.* It is composed of the usual two layers, an inner circular and an outer longitudinal one.

E. STRUCTURE OF THE KIDNEY

The kidney, like the liver, is a secreting gland and is composed mainly of epithelial cells. Examine a cross-section through the kidney of the frog. The flattened surface is ventral; the rounded surface dorsal. The lateral edge is more pointed than the medial and contains sections of a large vein, the renal portal vein, and of the mesonephric duct. In the middle of the ventral surface will be seen in some sections a yellowish or orange mass of epithelial cells, the *adrenal gland,* and in this region there are also usually present sections of large blood vessels.

1. The tubules.—The kidney is composed chiefly of extremely long tubular glands, called the *uriniferous tubules,* which are much coiled and twisted. Because of this coiling, the tubules appear in section as circles, ellipses, crescents, etc. Each tubule possesses a large and distinct central cavity and is composed of a single layer of cuboidal epithelial cells, whose function is the extraction of nitrogenous and other non-gaseous wastes from the blood. Note the numerous blood vessels between the tubules and the connective tissue covering the surface of the kidney and extending between the tubules.

2. The renal corpuscles.—In the ventral part of the section of the kidney note little rounded masses, the *renal corpuscles* (*Malpighian bodies*). Examine one with the high power. It consists of a dense network of capillaries called a *glomerulus* attached by a stalk through which an artery enters and a vein leaves. The glomerulus is covered with a delicate membrane which also forms the wall of the small cavity containing the glomerulus. This membrane, known as *Bowman's capsule,* thus has the shape of a hol-

low ball with one wall pushed in, the cavity of the pushed-in part containing the glomerulus. Each Bowman's capsule is the beginning of a uriniferous tubule, which leads away from the capsule opposite the point where the glomerulus is attached and after pursuing a winding course in the kidney enters a collecting tubule which opens into the mesonephric duct. Look over different renal corpuscles and try to find one which shows the continuity of Bowman's capsule with a tubule.

Draw a renal corpuscle and adjacent tubules.

F. STRUCTURE OF THE SPINAL CORD

The spinal cord and other parts of the central nervous system are considerably different in histological structure from other organs. Examine a section through the spinal cord of the frog. It consists of a central region, the *gray matter*, composed mainly of nerve-cell bodies, and a peripheral region, the *white matter*, composed of nerve-cell processes. In the center of the gray matter is a small canal, the *central canal*. The gray matter is trapezoidal in shape, its four corners being known as the *dorsal* and *ventral columns* of the gray matter. The ventral columns are considerably broader than the dorsal columns and contain the large motor cells studied in the preceding chapter. The spinal cord is nearly separated into two symmetrical halves by the *dorsal* and *ventral fissures* which extend from the middle of the dorsal and ventral surfaces toward the central canal. The dorsal fissure is shallow and is continued toward the central canal by a narrow *septum*, composed of loose tissue. The ventral fissure is much deeper and incloses an artery, the ventral spinal artery.

The white matter consists mostly of cross-sections of nerve fibers, which are the processes of the cells of the gray matter. The white matter also contains scattered cells which are mainly the cells of the *neuroglia*, a peculiar kind of connective tissue found only in the nervous system. The neurologia forms a network supporting the nervous structures.

Surrounding the spinal cord is a sheath, the *pia mater*, composed of connective tissue and containing numerous blood vessels. Some sections may show the roots of the spinal nerves springing from or at the sides of the section of the cord.

Draw the section of the cord showing its general features.

V. THE PROCESS OF CELL DIVISION

All cells arise from pre-existing cells by a process of *division*. In some cases this is a simple or *direct* division, in which a constriction (groove) appears on the surface of the cytoplasm and gradually deepening cuts nucleus and cytoplasm into two parts. But in the vast majority of cases, the cell divides by a complicated process, known as *indirect division, karyokinesis*, or *mitosis*. In mitosis, both the nucleus and cytoplasm are involved in a complex and remarkable behavior.

In order to demonstrate the process of mitosis to students it is necessary to select favorable materials in which division is proceeding rapidly and in which the cells are of reasonable size. There are not many such materials readily available. Parts which are growing rapidly and developing eggs furnish the most suitable objects. In the following account several such materials are described.

For convenience in description, mitosis is generally divided into several stages. The student should understand that it is a continuous process which in life occupies generally one to three hours.

A. MITOSIS IN THE EGGS OF ASCARIS

The *developing eggs* of any animal constitute favorable material in which to study the mitotic process because they are dividing rapidly. The student should understand that after an egg has been *fertilized* by a *spermatozoon* it divides in a very short time and these divisions are repeated at frequent intervals until a mass composed of a large number of cells is produced. This mass differentiates into a new animal. During these division stages of the developing egg then we may confidently expect to find at least some cells in the process of mitosis.

Ascaris megalocephala is a round worm (Phylum Nemathelminthes) parasitic in the intestine of the horse. Like many parasites it produces a great abundance of eggs. These eggs are favorable objects for the study of mitosis because they are of fair size and their chromosomes are large and few in number. The eggs are produced as in animals in general in the ovaries and thence pass down into the oviducts. The oviducts of Ascaris are very long and coiled and during this passage along the oviducts the eggs are fertilized by sperm injected into the oviducts during sexual union with a male Ascaris. After being fertilized the eggs continue to pass along the oviducts and begin to develop. This means that they divide to produce a number of cells. These divisions are of the mitotic kind and hence these developing eggs of Ascaris serve as objects for the study of mitosis. By cutting longitudinal slices of the oviducts in the region where the eggs are developing, we may expect to find stages of mitosis.

Before beginning this study, the student should first get clearly in mind that we are dealing with a fertilized egg. This means that a spermatozoon has entered the egg. Now as the egg has a nucleus of its own (being a cell) and as the sperm has also a nucleus of its own (being likewise a cell) a fertilized egg just after fertilization necessarily contains two nuclei, its own nucleus and the sperm nucleus. This condition does not last

very long as these two nuclei soon fuse into one and we can then no longer tell that this single nucleus was formed from two. But before the first division the fertilized egg does possess these two nuclei. The student should understand that this fact has nothing to do with mitosis but is the result of fertilization. After the first division, the cells of the developing embryo are each provided with but one nucleus as in ordinary cells.

Examine the slide of Ascaris with the low power. As already explained, the slide bears longitudinal sections of the oviduct of Ascaris. Note that each slice is bounded externally by a layer of epithelial cells on each side; these are the walls of the oviduct. The wide cavity of the oviduct is filled with a large number of round objects, each of which is an egg of Ascaris. Examine an egg with the high power and get a clear idea of what you are looking at. Each egg is surrounded by a thick *shell*. Inside the shell is a cavity containing the spherical egg which is much smaller than the cavity so that a considerable space is left between the egg and the shell. The egg is a round body highly granular with many spaces (vacuoles) in its cytoplasm. In many cases the egg will already have divided and two or more cells in close contact with each other will be found inside one eggshell.

Focus your attention on the eggs, disregarding the shells. Examine the eggs with your highest power and try to find the following stages of mitosis. Considerable searching among different eggs cells is necessary to find any one particular stage, as not many eggs are likely to be in that one stage and, further, the egg may not be cut in the right plane to show the mitotic process to advantage. Consider that each egg is a sphere and that the section on the slide is much thinner than the diameter of this sphere. An egg then may happen to be cut in any plane. The plane may not pass through the mitotic figure at all or may pass only through a piece of it. Obviously only those eggs in which the plane of section happens to be parallel to the plane of the mitotic figure are suitable for the study of some stages. Look for suitable eggs in each case. Avoid drawing eggs which show only a piece of the mitotic structures. Do not expect the real objects you see on the slide to look just like the diagrams of mitosis in your textbook.

1. Resting stage.—This name is given to any cell which is not in process of division; such a cell, of course, is not actually resting but is carrying on the usual metabolic functions. It is resting only with regard to the process of division. The fertilized eggs of Ascaris are in the resting stage between the process of fertilization and the beginning of the first division and also in the interval between all successive divisions. As already explained, the egg in the first case, that is, just after fertilization, will contain two nuclei, its own and the sperm nucleus. Eggs which have already divided and are preparing for new division contain a single nucleus. In either case, look for cells with regular ordinary nuclei like those you have already seen in other cells. Such nuclei have the form of a rounded or an oval body, filled with an invisible fluid, and containing a linin network on which are strung the deeply stained chromatin granules. Note granular vacuolated appearance of the cytoplasm.

Draw, showing structure of nucleus and cytoplasm Omit eggshell. Imitate the appearance of the cytoplasm by stippling with the point of the pencil.

2. Spireme stage or early prophase.—The onset of mitosis is revealed by a change in the chromatin of the nucleus. This begins to thicken and to mass into larger

granules. Meantime there appears in the cytoplasm in contact with the nucleus a dense spherical collection of granules which is the beginning of the *aster*. In the center of the aster is a black granule, the *centrosome*. Draw.

The chromatin of the nucleus continues to thicken and condense and finally takes the form of a long coiled thread which fills the nucleus. This thread is called the *spireme*. As we are dealing with sections the whole of the spireme cannot be expected to appear in any one section but only pieces of it. The nucleus in sections of this stage then appears filled with wormlike pieces of chromatin. The centrosome and aster divide.

3. Middle prophase.—The chromatin of the nucleus has completed the formation of the spireme. The aster has divided into two asters and in the center of each aster is seen a black granule, the centrosome. Each aster consists of a dense sphere of granules around which the granules of the cytoplasm are arranged in the form of rays. The two asters move apart and finally take up positions at opposites sides of the nucleus. In the fertilized egg about to undergo its first division there will be seen two nuclei with spiremes, the egg nucleus and the sperm nucleus; these two nuclei are in contact and an aster is present at each end of the plane of contact. In cells which have already divided, the single nucleus with its spireme is situated in the center of the cell with the asters at one side of it or at opposite ends of it. Draw a cell in middle prophase.

4. Late prophase.—The spireme thread breaks up into a number of threadlike bodies, the *chromosomes*. There are four of these in Ascaris. (It is probable that this division into chromosomes occurs very early in the formation of the spireme or may be present from the start.) The nuclear membrane has disappeared so that the four chromosones lie free in the cytoplasm in the center of the cell. They stain very deeply and are usually **U**- or **V**-shaped. Meantime the asters have reached positions at the opposite ends of the cell. Delicate rays or fibrils extend between them and are called the *spindle*. The whole structure now visible, asters, spindle, and chromosomes is named the *mitotic figure*. In order to see this figure in its entirety it is necessary to find a cell in which the plane of section is parallel to the plane of the spindle. If cut at right angles to the plane of the spindle the four chromosomes are seen lying free in the cytoplasm. Draw, showing the chromosomes in the cytoplasm.

In the final stage of the prophase, sometimes called *mesophase*, the chromosomes gather in the center of the spindle, forming a band (really a circle) of chromosomes known as the *equatorial plate*. The mitotic figure is now fully developed and symmetrically placed in the cell with an aster at each of its poles and the spindle fibers extending between them, spread wide apart at the equatorial plate and converging to each pole. Find a cell in this stage cut parallel to the plane of the spindle and draw, carefully showing asters, centrosomes, spindle fibers, and chromosomes.

5. Metaphase.—This stage is like the end of prophase except that each chromosome develops a longitudinal split along its center; or perhaps more correctly speaking, a split previously present now becomes plainly visible. The split in the chromosomes is not always easy to see in Ascaris.

6. Anaphase.—The two halves of each chromosome separate and one half moves to one pole, the other half to the other. In this migration the ends of the **V**-shaped chromosomes always point toward the center of the spindle, so that at this stage the cell

contains two groups of chromosomes each looking something like the top of a palm tree. The delicate parallel fibers of the spindle are seen stretching between the two groups of chromosomes. The movement of the chromosomes seems to be caused by the pull of the spindle fibers which are attached to them. A constriction or groove is appearing in the surface of the cell opposite the center of the spindle.

7. Late anaphase.—This is like the preceding except that each group of chromosomes has reached an aster. The constriction is deepened. A line, the future *cell membrane,* may be appearing across the cell through the center of the spindle. Find a cell in any stage of anaphase which is cut parallel to the plane of the spindle and draw.

8. Telophase.—Each group of chromosomes is becoming unrecognizable as such and is reverting to the condition of an ordinary nucleus. The membrane across the cell is complete and two cells like the original one have been produced. These remain together for they will by further divisions form a new worm.

B. MITOSIS IN THE ROOT TIPS OF PLANTS

The root tips of plants grow very rapidly in some cases and hence are favorable places for finding stages of mitosis, for in such growth new cells are continually formed by the division of cells already present. For the study of the mitotic process in plant root tips, longitudinal sections are made through the tip. Examine such a section under low power. Note the *root cap* or loose material composed of old and hardened cells which forms the extreme tip and which is pushed ahead of the growing region. The growing region, where the dividing cells are situated, is a little back from the root cap. Observe that the cells of the growing region are arranged in longitudinal rows.

Study the cells with the high power. Each is somewhat squarish in outline with a definite cell wall, a large spherical nucleus, and granular cytoplasm. Try to find by searching among the different cells the following stages of mitosis.

1. Resting stage.—This name is applied to any cell which is not in process of division; such a cell is, of course, not really resting but is carrying on the ordinary activities. In this stage in a root-tip cell, the nucleus presents the usual appearance. It is a spherical body filled with the usual linin network bearing granules of chromatin. There are one or two large spherical granules in the nucleus, which are known as *nucleoli.* Draw.

2. Early prophase or spireme stage.—The beginning of mitosis is marked by an enlargement of the chromatin granules which now appear thicker and more dense than before. This process continues until the chromatin takes the form of a thick thread. Finally the whole nucleus is filled with a long coiled thread of chromatin which is called the *spireme.* Owing to the fact that the sections are generally thinner than the diameter of the nucleus the entire spireme will not appear in any one section but only pieces of it. Consequently in sections through the spireme stage the nucleus generally appears filled with wormlike pieces of the spireme. Draw.

3. Middle prophase.—The nuclear membrane and nucleoli have disappeared so that the chromatin lies free in the cytoplasm. The spireme has broken up into a number of definite threadlike bodies, the *chromosomes.*

4. Late prophase.—The chromosomes arrange themselves in a definite plate in the center of the cell, called the *equatorial plate.* In the meantime there has appeared in the cytoplasm a structure, called the *spindle,* composed of fibrils. This spindle is so named because it has the shape of a spindle, that is, it is widest in the center and its fibrils converge to points, called the *poles* of the spindle, at each end. The equatorial plate of chromosomes is arranged in the center of the spindle. Asters and centrosomes, characteristic of animal cells, are lacking in most plant cells. Find a late prophase stage in which the cell is cut parallel to the plane of the spindle and **draw.** This stage is sometimes called the *mesophase.*

5. Metaphase.—This stage is like the late prophase except that each chromosome has developed a longitudinal split. It is difficult to see this split.

6. Anaphase.—Each chromosome separates into two longitudinal halves. One half of each moves to one pole of the spindle and the other half to the other pole. In this movement the ends of the threadlike **V**-shaped chromosomes point toward the center of the cell, so that each group of chromosomes looks something like the top of a palm tree. Each group of chromosomes continues to move away from the center of the cell until it reaches one of the poles of the spindle. Between the two groups of chromosomes are seen the spindle fibers. **Draw.**

7. Telophase.—Having reached the poles of the spindle, each group of chromosomes condenses into a mass in which the individual chromosomes are no longer visible. A faint line, the new *cell wall,* is seen across the middle of the cell and seems to be composed in part of the central parts of the spindle fibers. At the end of the telophase the mass of chromatin gradually transforms into an ordinary nucleus, like that with which we started, and the cell wall is completed. **Draw** a cell in telophase.

C. MITOSIS IN THE TESTIS OF THE CRAYFISH

The reproductive organs of animals at the time of sexual activity furnish favorable places for the study of mitosis as the cells are dividing rapidly to produce the sex cells. The testis of the western crayfish is generally used for this purpose, owing to the large size of its cells.

Examine under low power a section through the testis of the crayfish. The section consists of round masses each of which is a *tubule* of the testis. At the periphery (surface) of each tubule is a layer of cells which are not concerned in the production of sex cells. The cells which fill the rest of the tubule are the ancestors of the sperm and at the proper season of the year are actively engaged in mitosis. All of these cells in any one tubule will be found to be in about the same stage of mitosis.

1. Resting stage.—This is the name given to cells not in process of division. Such a cell looks like the cells we have already studied. In the testis of the crayfish resting cells may be found in the smallest tubules, but most of the cells are in mitosis. Locate under low power one of the smallest tubules and study its central cells under high power. Each of these cells is somewhat hexagonal in form with granular cytoplasm. It contains a large round nucleus in which is the usual network with chromatin granules.

2. Early prophase or spireme stage.—For this and subsequent stages study the larger tubules. The beginning of mitosis is recognized by a thickening of the chromatin

granules of the nucleus. This process continues until the chromatin has condensed into filaments. The nucleus becomes filled with a tangle of chromatin filaments. This tangle is called the *spireme*. Each chromatin filament in it is called a *chromosome*. **Draw.**

3. Middle prophase.—The nuclear membrane disappears, setting the numerous chromosomes free in the cytoplasm. They have become shorter and more rodlike. A fibrillar structure is dimly seen appearing in the cytoplasm.

4. Late prophase.—The fibrillar structure has developed completely and is now seen to consist mainly of a beautiful bundle of fibrils, called the *spindle*. The spindle is so named because it has the shape of a spindle, that is, it is widest in the middle and converges to points at each end, called the *poles* of the spindle. At each pole of the spindle is seen a rounded mass, which is named the *central body*. From this a few fibrils radiate into the cytoplasm. Central bodies and rays constitute the *asters*, but these are poorly developed here as compared with other animal cells. The chromosomes now appear as rodlike bodies and are arranged in a plate, called the *equatorial plate*, throughout the center of the spindle. The entire structure, asters, spindle, and chromosomes is known as the *mitotic* or *karyokinetic figure*. To see it in its entirety, find a cell in which the plane of section is parallel to the plane of the long axis of the spindle. **Draw.** Find a cell cut at right angles to this and note the equatorial plate of chromosomes occupying the center of the cell. **Draw** this.

5. Metaphase.—This stage is like the late prophase except that the chromosomes are obviously divided in two and appear in two bands across the center of the spindle. **Draw.**

6. Anaphase.—The two groups of chromosomes draw apart. One group moves toward one pole of the spindle, the other to the other pole. The spindle fibers are plainly seen stretching between the two groups. At the end of the anaphase, each group has reached a pole. **Draw** a cell cut parallel to the long axis of the spindle, showing the anaphase stage.

7. Telophase.—At each pole each group of chromosomes condenses into a mass of chromatin. Across the center of the cell a new cell membrane is seen forming. **Draw.** Subsequently a new nucleus is formed in each cell around the chromatin mass, which reverts to the resting condition.

The curious oval bodies which may be noticed in some of the tubules of the testis are the completed spermatozoa. The spermatozoa of the crayfish are very different in form from those of most other animals.

47-50 Embryology
starfish ; frog

VI. GENERAL EMBRYOLOGY

The egg cell after the sperm cell has penetrated it (process of *fertilization*) enters on a series of changes which are collectively called *development*. The study of these changes constitutes the subject of *embryology*. After the egg has begun to develop it is called an *embryo*. The embryo may complete its development within the eggshell or egg membrane, or within the parent body, finally hatching or emerging in a form practically like the adult except as to size and sexual maturity. In many groups of animals, however, the embryo becomes free at an early stage and is then known as a *larva*. The larva is usually very unlike the adult in appearance and frequently changes into the adult form by a sudden transformation known as a *metamorphosis*.

The first stages of development of all animals are more or less similar. Thereafter, the development of different groups of animals diverges, at an early period in distantly related animals, at a late period in closely related forms.

The first stages of the development of animals consist in the repeated division of the egg by *mitosis* until a large number of cells is produced. This process is known as *cleavage* or *segmentation of the egg*. It leads to a stage known as the *blastula*, which is followed by another stage, the *gastrula*. *Differentiation* of the parts and cells of the embryo then begins and continues until the adult anatomy is attained.

A. DEVELOPMENT OF ECHINODERMS

The echinoderms are a group of animals, including such forms as starfishes and sea-urchins (specimens of these should be at hand for demonstration), which are often employed to illustrate embryonic development because their eggs and sperm are obtained easily and in large numbers and because their early development is typical. Examine slides showing development of the starfish or sea-urchin or sand-dollar egg. Note that the objects on the slides are the *entire* eggs and embryos, *not* sections. Each egg and early embryo is surrounded by a membrane, the *fertilization membrane*, which separates from the egg at the time of fertilization and is a sign that fertilization has occurred. Eggs may be noticed on the slides which have a large clear nucleus; these are eggs that have failed to mature and hence were not fertilized.

1. **Two-celled stage.**—The egg first divides by mitosis into two cells. Find an example on your slide and draw in outline only.

2. **Four-celled stage.**—A second division then occurs at right angles to but passing through the same axis as the first. **Draw.**

3. **Eight-celled stage.**—Each cell of the four-celled stage divides in two transversely at right angles to both of the previous divisions, producing eight cells in two tiers of four cells each. Are the eight cells of equal size? **Draw.** Avoid drawing specimens in which the eight cells have become displaced owing to the pressure of the cover glass.

4. Later stages of cleavage.—The process of division continues until a considerable number of cells is produced. Meantime, a central cavity appears between the cells. **Draw.**

5. Blastula stage.—After cleavage has continued for some time, an embryonic stage known as the *blastula* is reached. The blastula is a spherical hollow object with a wall one cell layer thick (more than one layer in some animals). The central cavity of the blastula is called the *blastocoel* or *segmentation cavity*. Find a blastula and focus so that you obtain an *optical section* (i.e., focus on the center of the object so that it looks as if you had actually made a section through its middle). In such a focus the blastula appears as a circular layer of cells surrounding the large blastocoel. Are the cells all of the same size? **Draw the optical section.**

The blastula of echinoderms develops cilia on the surface, escapes from the fertilization membrane, and swims about free in the water as a *larva*.

6. Gastrula.—The blastula soon develops into another embryonic stage, called the *gastrula*. The gastrula marks the beginning of differentiation, that is, the setting off of part and cells of the embryo for particular functions. It should be understood, however, that cell division continues for a long time. The gastrula arises from the blastula by the pushing in of one wall of the latter just as if one should thrust one's finger into the side of a rubber ball. The gastrula appears on the slide as an oval body, containing a central darker projection. Find one and obtain an optical focus on it. Note the outer wall, called the *ectoderm*, one cell layer thick. It surrounds a cavity, the remains of the blastocoel, in the center of which is a finger-shaped projection. This projection is the *primitive intestine*, or *archenteron*, formed by the in-pushing (*invagination*) of a portion of the wall of the blastula. The layer of cells composing the archenteron is named the *entoderm*. The opening of the archenteron to the outside is the *blastopore*. We see that in the gastrula there is a differentiation into a food-digesting part, the archenteron, and a surface protective layer, the ectoderm. **Draw the gastrula** in optical section.

B. DEVELOPMENT OF THE FROG

Living material should be used if available. Otherwise preserved material should be employed. Read in texts and study figures of the development of the frog. Remove the jelly in each case before attempting to study the egg or embryo.

1. The egg.—The egg of the frog is much larger than the egg of echinoderms because it contains a considerable quantity of *yolk*, a semifluid food material composed chiefly of proteins and fats. The egg is surrounded by a *jelly* secreted by the oviducts of the frog at the time of laying. This jelly causes the eggs to adhere in masses, which in nature are attached to sticks, etc., in ponds and streams or may float on the surface. The egg is half black and half white, the black half constituting the *animal hemisphere* consisting chiefly of protoplasm, while the white half or *vegetal hemisphere* contains most of the yolk. The center of the animal hemisphere is the *animal pole* and becomes the anterior end of the embryo; the center of the vegetal hemisphere is the *vegetal pole*.

2. Cleavage stages.—Owing to the presence of the yolk, which is inert, non-living material, the vegetal hemisphere divides more slowly and produces larger cells than the animal hemisphere. Study eggs in cleavage with the low power or a hand lens. To see

the cleavage furrows clearly it is necessary to shine a strong light on the eggs. If available, **draw two-, four-, and eight-cell** stages. Note in the eight-cell stage, the large size of the vegetal as compared with the animal cells. Six-cell stages are not uncommon owing to the delay of cleavage in the vegetal half.

3. **Blastula.**—After numerous cleavages a blastula is produced consisting of numerous very small black cells and less numerous larger white cells. **Draw** from surface. Then with a sharp knife bisect the blastula by a cut which cleaves the black and white hemispheres or use a prepared section through the blastula. Study the section with hand lens or low power. Note that the wall of the blastula is two or three layers thick, that the cells of the animal or *dorsal* half are more numerous and smaller than the cells of the vegetal or *ventral* half, that the ventral wall is thicker than the dorsal, and that the blastocoel is small and displaced dorsally. **Draw the section.**

4. **Gastrula or yolk-plug stage.**—The gastrula is produced in part by an *invagination* process as in the echinoderm egg. At one place at the boundary of animal and vegetal hemispheres, there is a turning in of cells of the vegetal hemisphere. Gastrulation is also accomplished in part by the growing down of the animal cells over the vegetal cells. The end result is the same as in the echinoderm embryo, that is, part of the original external surface now projects into the interior as the *entoderm* and forms the *primitive intestine* or *archenteron*. The opening of this to the exterior is the *blastopore*. Part of the entoderm cells project from the blastopore for some time, forming a white mass, the *yolk plug*, which fills the blastopore. **Draw** from external view, showing blastopore and yolk plug. Then bisect the gastrula by a cut through the yolk plug and center of the animal hemisphere or examine prepared sections of this stage. Study with low power and compare with figures in texts. Note outer *ectoderm*, in-turned mass of vegetal cells forming the *entoderm*, remains of *blastocoel*, *yolk plug* filling the *blastopore*, and *cavity of the archenteron* between the yolk plug and the *dorsal lip* of the *blastopore*. **Draw the section.**

5. **Origin of the central nervous system; neural fold stage.**—Examine embryos of this stage and note a central groove running along the black hemisphere with a fold on each side of it. The central groove marks the median dorsal line of the embryo. The pair of folds are the *medullary* or *neural folds*. The blastopore which represents the future anus of the animal is reduced to a small hole. **Draw.** The neural folds later come in contact and fuse in the median dorsal line forming a longitudinal tube which is the *central nervous system*.

6. **Origin of the mesoderm and the coelome.**—Study a cross-section of a stage just after the closure of the neural folds. The cross-section is roughly pear-shaped, the narrow end being the *dorsal* side of the embryo. The surface of the embryo is formed of a layer of uniform width, two or three cells thick. This is the *ectoderm*, destined to become the epidermis of the skin. In the mid-dorsal line, just under the ectoderm is an oval mass of cells with a central elongated cavity. This is the cross-section of the *neural tube* or *central nervous system* which originates as described above. Just ventral to the neural tube is a circular mass, the *notochord*, which arises by an upfolding of the mid-dorsal wall of the intestine It is a long slender rod, around which the vertebrae later develop. Ventral to the notochord appears the *primitive intestine* or *archenteron*, a

large nearly circular mass of ill-defined cells, with a thin dorsal wall, thick ventral yolk-filled wall, and a small cavity near the dorsal wall. This intestine is the *entoderm* and it becomes the lining epithelium of the adult digestive tract. Between the ectoderm and the entoderm lies the third embryonic layer, the *mesoderm*. It consists of a large triangular mass of cells on each side of the neural tube, and a layer of cells extending from this ventrally on either side of the intestine. The mesoderm mass of each side generally shows a central split, which is the *coelome*. The mesoderm to the outer side of the split, next to the ectoderm, then represents the *parietal* layer of the peritoneum and some of the connective tissue of the body wall. The mesoderm to the inner side of the split, next to the entoderm, is destined to give rise to the connective tissue and muscular layers of the wall of the digestive tract, the *visceral* peritoneum, and the mesenteries. The triangular masses of mesoderm at the sides of the neural tube produce the vertebral column, nearly all of the voluntary muscles, and the dermis of the skin. The mesoderm immediately ventral to each triangular mass is the source of the urogenital system. **Draw the section.** Do not attempt to put in individual cells as these are not very distinct.

7. **Later embryonic development.**—At about the time of formation of the neural folds the embryo begins to elongate. It then presents *anterior* and *posterior* ends, a flattened *dorsal* surface, and a rounded *ventral* surface. Examine an embryo after the closure of the neural folds. Remove it from the jelly. It is elongated in form. Along the mid-dorsal surface runs a more or less obvious ridge, which is the *central nervous system*. The anterior end, the future *head*, is slightly enlarged, and presents a swollen area on each side, which is called the *sense plate*. This becomes part of the head, including upper and lower jaws as well as other structures. On the sides of the body back of the sense plates are three elevations with grooves between them. These are called the *gill plates*. From the elevations *gills* later grow while the grooves become *gill slits*, that is, openings from the pharynx to the outside for respiratory purposes. On the ventral surface of the anterior end of the embryo is seen a **U**-shaped groove bounded by raised lips. This is called the *sucker*. At the posterior end of the dorsal surface is a projection, the *tail bud*. Below this is a round hole, the *anus*, derived from the blastopore. **Draw** from side.

A little later, the tail bud is seen elongating, so that head and tail of the embryo are now distinct. The gill plates are more conspicuous. The middle part of the **U**-shaped sucker begins to disappear.

8. **Larva at hatching.**—The time between the laying and the hatching of the eggs of frogs varies with the species and the temperature of the water. Development will not occur at all at too high or too low temperatures; but between these extremes, the rate of development is more rapid the higher the temperature, up to a certain point. The eggs of our common leopard frog, *Rana pipiens*, hatch in one to three weeks, at moderate temperatures.

Many animals at hatching closely resemble their parents except in size. But the creature which emerges from the egg of the frog is not at all like a frog. *Larva* is the name given to a young free-living stage of an animal, when such stage differs considerably in appearance from the adult. The larvae of the frog are commonly known as *tadpoles* or *polliwogs*.

Examine a newly hatched frog tadpole. It is a small slender black creature, seven to eight mm. long. It consists of a somewhat stout body and conspicuous tail. On examination with a hand lens or the low power of the microscope, the following structures can be seen. On the ventral surface of the rounded head are situated two oval pits with raised lips. These are the suckers and are formed from the original **U**-shaped sucker by the disappearance of its median portion. In front of the suckers is seen the *mouth* opening. Springing from the sides of the head are a few branched projections, the *gills*. These have grown out from the gill plates previously noted. In front of the gills may be seen on each side a slight rounded elevation which is the location of the future *eye*. The *tail* is very broad dorso-ventrally, but narrow laterally. It consists of a central thicker axis, which contains the *notochord* (embryonic representative of the vertebral column) and of the thinner *tail fin* which borders this. Draw tadpole from ventral view.

If possible, observe living newly hatched tadpoles. Note use of the suckers in clinging to objects. It is said that the suckers do not cling by suction but by the secretion of a sticky material. Observe behavior of the tadpoles when the dish is jarred and method of swimming. The body of the tadpole is ciliated at this time.

9. Changes during the first week after hatching.—In order to observe the development further in the living state the tadpoles must be fed. They still contain at hatching some yolk in the walls of the intestine but this does not suffice for very long and if one desires to rear the tadpoles food should be provided. (See Appendix.)

During the first few days after hatching marked external changes take place. The most important of these is the appearance of a fold around the posterior part of the head. This fold, called the *operculum*, grows backward over the gills which it incloses and fuses with the body wall at all points except for a small area on the left side, which remains as a spout for the exit of the respiratory current. While this change is occurring the body is growing rounder and stouter and through the ventral body wall the coils of the *intestine* can be seen. The suckers gradually disappear. The *mouth* elongates and becomes provided with *lips*. The two *eyes* are seen on the dorsal surface of the broad head. The *tail fin* is broader and more transparent.

10. External structure of the developed tadpole.—The tadpole undergoes no further marked external changes for a long time but if fed grows rapidly in size. Its color becomes lighter. It has a stout oval body and a large tail, with a central dark axis and a wide thin tail fin.

Note following structures. On the dorsal surface of the anterior part of the body are the two *eyes*. In front of them are located the two *external nares*. On the left side of the body (rarely on the right side) is seen a spoutlike projection, the *spiracle*, whose mode of formation was noted above. On the ventral surface near the anterior end is the elongated *mouth*, considerably different in structure from that of the adult. Inside the mouth opening are the *jaws*, each of which is sheathed with a *horny* coat bearing *teeth*. On the inner surface of the lips are also rows of *horny teeth* and on their outer surfaces a fold bearing a row of soft projections or *papillae*, probably organs of taste and touch. Note the large coiled *intestine* seen through the ventral body wall. In the axis of the tail note the zigzag *muscle segments* or *myotomes*, bordered above and below by the thin *tail fin*. In the ventral part of the fin just posterior to the junction of trunk and tail the end of the intestine is seen opening to the exterior by the *anus*.

On the dorsal surface of the body, extending also posteriorly along the tail, may be noticed (best in living specimens) light-colored or silvery dots arranged in curving lines. These lines constitute the *lateral line system* and each dot is a sense organ of this system. Each dot consists of a group of sensory cells, slightly elevated above the surface of the skin. The function of the lateral line system appears to be the perception of vibrations in the water. This system is found in fishes and the aquatic stages of Amphibia.

It may be emphasized at this point that the frog tadpole possesses several structures characteristic of fish, such as gills, the lateral line system, and the arrangement of the muscle segments in the tail. This similarity of structure furnishes an illustration of one of the most fundamental and general laws of embryology, namely, that animals in their development pass through stages that resemble in their structural features animals lower in the scale of life than themselves. This principle is called the *law of recapitulation*. But one reasonable explanation of this principle has been found by biologists, namely, that the stages in the development of an animal resemble lower forms because these forms were its ancestors. The tadpole of the frog possesses many features characteristic of fish because frogs are descended from fishes.

In living tadpoles note use of the tail in swimming, method of employing the horny jaws and teeth in feeding, and respiratory current. This is a current of water entering through the mouth and passing out through the spiracle. It can be observed by watching particles near the spiracle.

Draw a tadpole from above. **Draw** enlarged view of mouth.

11. Appearance of the hind limbs.—After a time a pair of buds is seen developing at the sides of the junction of trunk and tail. These grow out and are seen to be the hind limbs. Eventually they come to have the shape typical of the adult frog. Does the tadpole use them at first? later?

12. Dissection of a tadpole with hind limbs.—Remove the entire ventral wall of the tadpole and study the internal organs. The posterior part of the body cavity is occupied chiefly by the very long coiled *intestine*. In front of this may be seen the *fore limbs* if the tadpole is sufficiently advanced in its development; otherwise they are not very noticeable.

Study the posterior viscera first. As already noted, one sees at first little but the voluminous *intestine*. The much greater length of the tadpole intestine as compared with that of the adult is due to the vegetarian habits of the tadpole. It is generally true that vegetable feeders possess long intestines, carnivorous animals, short intestines. By moving the intestine about, other organs with which we have already become familiar in the adult frog are revealed. The *liver* lies chiefly to the right side and is lobed as in the adult. The *gall bladder* is proportionately larger than in the adult. The *stomach* is not very well developed but the *pancreas* is obvious. *Fat bodies* and *lungs* are present. By pushing the intestine to one side, the *kidneys,* proportionately very much larger than in the adult, will be found against the dorsal wall.

The anterior part of the body contains some structures which are not found in the adult frog. The *fore limbs* have already been noted or if they are still in a young stage of development, will be found later. In the median ventral line is situated the heart. To

each side of the heart are seen objects that we have not hitherto met with. These are the three pairs of *gill arches* on which are borne soft filaments, the *gills*. With a forceps probe among the gill arches and determine that they are arch-shaped structures, three on each side, fastened at their dorsal and ventral ends, free in their middle regions. The spaces between the gill arches are called the *gill slits*. Pass an instrument through the mouth opening and determine that it can emerge through the gill slits, showing that there is a continuous passage from the mouth out through the gill slits. The gill arches are in fact structures in the wall of the pharynx and the gill slits are openings from the cavity of the pharynx through its wall to the cavity covered by the opercular fold. This cavity, as already noted, opens to the exterior by way of the spiracle. The whole apparatus constitutes the respiratory mechanism of the tadpole and is *very similar to that of a fish*. Water taken in at the mouth passes through the gill slits, over the gills, and out of the spiracle. During this passage the usual gaseous exchange takes place through the thin walls of the gills.

The fore limbs develop under the operculum as buds from the body wall just back of the dorsal end of the third gill arches. They will be found here in all tadpoles that have small hind legs. As development proceeds they grow larger and finally in tadpoles with large hind legs the fore legs are conspicuous objects and partly conceal the gill arches.

Draw your dissection.

13. **Emergence of the fore limbs.**—As already noted the fore limbs develop in the opercular chamber, and are concealed from external view until nearly the end of the larval development. They then emerge to the exterior. The left one appears first and projects through the spiracle. A little later the right one breaks through the opercular wall. While these changes are occurring the body of the tadpole, having now reached its full size, begins to take on the form and proportions of the adult frog.

Draw tadpole with two pairs of limbs.

14. **Metamorphosis.**—Very soon after the emergence of the fore limbs the tadpole rapidly changes into a young frog. Such a sudden change from a larval to the adult form is called a *metamorphosis*. A number of changes occur rapidly. The tail diminishes in size. The body assumes the shape of the adult. The mouth and jaws transform to the adult condition, the horny structures being shed. Eyelids develop on the eyes. The lateral line organs disappear. The gills are resorbed, and respiration is now wholly by means of the lungs, which, however, are used to some extent for some time before metamorphosis. The animal at this time must have free access to the air and will drown if kept under water. The tail is finally reduced to a stump and rapidly disappears altogether. When all of these changes are completed we have in place of the tadpole a miniature frog. It is no longer a vegetarian but feeds **chiefly** on insects. The time from hatching to metamorphosis is eight to ten weeks in the leopard frog. The actual metamorphosis occupies but two or three days.

15. **Distinguishing characteristics of the tadpoles of our larger frogs.**—

a) Leopard frog (*Rana pipiens*). Maximum length, three inches. Color, body mottled brown or olive above, belly cream with a bronze iridescence, tail fin very light, almost transparent, with fine scattered black specks. Eggs laid in March and April, at-

tached to submerged objects; metamorphosis two to three months later; tadpoles therefore not found in the fall.

b) Pickerel frog (*Rana palustris*). Under three inches in length. Body dark, tail fin opaque, dark, with dark splotches, belly as in leopard frog. Eggs laid in April and May, attached to submerged objects; metamorphosis in late summer or early fall of same year.

c) Green frog (*Rana clamitans*). Maximum length, three and one-half inches. Body and particularly tail mottled with large splotches, belly cream with no iridescence. Eggs laid mostly in June and July, floating; metamorphosis during the summer of the following year.

d) Bull frog (*Rana catesbiana*). May reach a length of six or seven inches. Of a general olive color with regular round black spots on body and tail fin. Eggs laid in June and July in a large floating mass; metamorphosis in summer two (or even three) years from the laying of the eggs.

Any large tadpole found in the fall or early spring is almost certainly that of the green frog or the bull frog.

C. GENERAL CONSIDERATIONS

From our study of embryology we learn that each multicellular animal arises from a single cell, the egg cell, which, stimulated to activity by the entrance into its substance of a sperm, starts on a process of development during which the multitude of structures found in the adult come into existence.

It is further evident that in the development of animals certain fundamental steps are involved. First, the egg divides by mitosis into a number of apparently similar cells. This process continues until a hollow ball of cells, the blastula, is produced. There then occurs the first step in differentiation, namely, part of the surface is brought into the interior, so that two layers of cells are now present, an outer layer, the *ectoderm,* and an inner layer, the *entoderm.* Owing to their different positions, these two layers have different relations to the external world, and hence must take on different functions. The ectoderm, being in contact with the environment, must necessarily receive stimuli and protect the animal from external conditions; hence it is destined for nervous and covering structures. The entoderm naturally takes on digestive functions, for food is essential to life and food cannot be utilized by the animal unless taken into its interior. This structural condition of two layers found in the gastrula stage of development is known as *diploblastic* (two cell layers) and, as we shall see, thousands of animals exist whose structure has gone no farther than this. However, it is obvious that no very great degree of complexity and differentiation can be attained in a gastrula; and a third (double) layer, the *mesoderm,* next arises between the other two. The greater part of the structures possessed by animals like the frog originate from the mesoderm. Animals whose structure is derived from three embryonic layers are said to be *triploblastic.* Further steps in the development of the higher animals are the formation of the *coelome* by a splitting of the mesoderm (other methods of formation of the coelome also exist) and the *segmentation* of the mesoderm.

VII. HEREDITY: MENDEL'S LAW

In the experiment on the life-cycle of the fruit fly (Drosophila) you were given a pair of flies which differed from each other in a single character, as long wings and short wings. (If blowflies were provided for that experiment, a pair of fruit flies will now be given to you and should be examined according to the directions under II, G.) The offspring resulting from such a pairing between unlike individuals are therefore *hybrids*, and it becomes a matter of great interest to find out what will be the appearance of the offspring, as we may then discover how the characters of animals behave in heredity.

A. FIRST HYBRID GENERATION

When the offspring appear note carefully the character of the wings (or other feature which was selected for the experiment). Are they all alike, or of two kinds, like the parents, or do they resemble one parent more than the other, or are they intermediate? What is the meaning of the terms "dominant" and "recessive" as applied to a pair of characters such as we are dealing with here (*A*)?

A fresh bottle with banana will be provided for each student into which he is to put one or two pairs of flies from his first generation and raise a second generation. The transference of the flies may be accomplished by dropping a small bit of cotton soaked in ether into the bottle and taking out the flies after they have become unconscious, or by holding a bottle over the end of the old culture bottle until a few flies have flown into it, then holding the bottle over the new culture bottle until they have flown out again. In doing this make use of the tendency of Drosophila to fly toward the light.

B. SECOND HYBRID GENERATION

In the second hybrid generation note again the character of the wings (or other feature selected) and determine the number of individuals with each kind of wing length. This behavior of a hereditary character is called *Mendel's law* or *alternative inheritance*. If we suppose that every female fly of the first hybrid generation gave rise to two kinds of eggs in equal numbers, one bearing the character "long wings" and the other the character "short wings"; and that similarly every male of the first hybrid generation produced two kinds of sperms in equal numbers; and that in fertilization all possible chance combinations occurred (i.e., each kind of sperm fertilizes both kinds of eggs), what kind and proportions of offspring would we expect mathematically? Thus if *L* is taken to represent the character "long wings" and *s* the character "short wings" we get as a result of algebraic multiplication the following possible combinations in equal numbers: *LL, Ls, Ls, ss*. What will be the external appearance of the flies containing each of these combinations? Will *LL* differ from *Ls*? Why? What then will be the ratio of long- to short-winged individuals in the second generation?

These experiments were first performed by a monk named Mendel, who made crosses between different varieties of peas in the garden of the monastery at Brünn,

Austria. He discovered that in such crosses the differences between the two plants that are crossed do not become permanently blended in the offspring but separate out again unchanged in the second generation. This behavior of the characters in crossing two different organisms is called Mendel's law, and Mendel's results have since been confirmed on a very large number of plants and animals. The conclusion which has been drawn from these experiments is that the phenomenon of heredity results from separate self-perpetuating units, called genes, which exist in the chromosomes of the eggs and sperms; that these genes remain separate in the cells of a hybrid organism even though the hybrid may appear externally to be a blend of the characters of its two unlike parents or may resemble only one of them; and that under proper circumstances, the genes may be made to separate out again unchanged. Any one external character of an animal such as the red eye of Drosophila is the resultant of the interaction of a number of genes with the cell cytoplasm through a series of physiological processes. Thus neither the genes nor their environment are alone responsible for the appearance of characters, but both interacting together are necessary.

VIII. PHYLUM PROTOZOA

The animal kingdom is divided up by zoölogists into large groups, called *phyla*. Each phylum includes an assemblage of animal forms which resemble each other in important anatomical characteristics. The lowest phylum of the animal kingdom is named *Protozoa* and includes the simplest known animals. The Protozoa are defined as animals that consist of but a single cell or of colonies of like cells. We have seen in our study of the frog that the frog is composed of cells, the cell therefore being the *unit of structure* of a complex animal, and that the frog develops from a single cell. We shall now see that numerous adult animals, the Protozoa, exist which consist of but a single cell but are able to carry on all of the functions necessary to life within the limits of this cell.

A. AMOEBA

1. General structure and locomotion.—Mount a few drops of solid material from the culture on a slide, cover, and examine with the low power. Cut down the light. Search the slide for an irregular granular sharply outlined object, apparently motionless. Ask the instructor whether or not you have an Amoeba or have him help you find one. Study the animal with both low and high powers. Observe that the Amoeba moves by putting out projections, called *pseudopodia*, from its surface and then flowing into these projections. This type of movement is designated as *amoeboid movement*. The protoplasm of the Amoeba is divisible into two regions, an outer clear layer, the *ectoplasm*, free from granules, and the central mass, the *endoplasm* (or entoplasm) filled with granules and food particles. Under the high power, watch the formation of a pseudopodium and determine how the ectoplasm and endoplasm behave in its formation. Does the Amoeba have a definite form and anterior and posterior ends?

Make five outline drawings of an Amoeba showing successive changes of shape. Indicate by arrows the direction of flow of the protoplasm in each pseudopod.

2. Special structures.—Find a specimen whose protoplasm is well spread out and not excessively granular and look with the high power for the following structures:

a) *Contractile vacuole:* Watch in the "rear" part of the animal for a spherical clear spot. At intervals it contracts and disappears, hence the name contractile vacuole. Watch and describe in your notes the contraction, and position and size of the vacuole when it reappears. Sometimes two or more vacuoles are present but they usually coalesce into one later.

b) *Nucleus:* This is a sharply outlined finely granular body in the neighborhood of the contractile vacuole, often in contact with it and of about the same size as the fully expanded vacuole. It contains *chromatin granules*, fine and evenly spaced. Determine the real shape of the nucleus by watching it as it rolls along in the endoplasm. Is it spherical? Occasionally specimens have two nuclei. The nucleus is difficult to see in small specimens.

c) *Food vacuoles:* The endoplasm usually contains particles or masses of digesting food which are usually inclosed in a drop of fluid called a *food vacuole.*

Make an enlarged drawing of the Amoeba and put into it all details of structure. Indicate the granular appearance by stippling with the pencil point.

3. **Activities.**—Can you observe any indications that the Amoeba is irritable, that is, perceives and responds to the varying conditions of its environment? For instance, what does it do when it comes in contact with an obstacle or when other animals strike against it? When jarred? If the Amoeba is in good condition with plenty of water in which to creep you may be able to observe the ingestion of food. If so, watch, sketch, and describe in your notes.

4. **General considerations on Amoeba.**—Does the Amoeba respond to and conduct stimuli, respire, excrete, digest and assimilate food, reproduce, etc.? Does it have any particular organs for these functions? What are the resemblances between Amoeba and the frog or man? What are the important differences? Do you think that man has any advantages over the Amoeba by reason of his greater complexity of structure? Disadvantages? To what stage of embryonic development do Amoeba and other Protozoa correspond? Give these questions careful thought and answer specifically in your notes.

B. EUGLENA

Euglena belongs to the group of Protozoa known as *Flagellata* or *Mastigophora,* commonly called flagellates, characterized by the possession of one to many long thread-like processes of the protoplasm, known as *flagella,* and used in locomotion.

1. **General form and movements.**—Examine the culture of Euglena. Although the individual animals are too small to be seen with the naked eye, the green color produced by masses of them is quite evident. Do the animals show any definite distribution in the culture with reference to light or air?

Place a small drop of the green material on a slide, cover, and examine with the low power. The small green objects are specimens of Euglena. Usually some of them are swimming about actively, others are more or less stationary, and still others may be rounded up into motionless balls (*cysts*). In those which are swimming about, note the peculiar swaying swimming movement characteristic of flagellates. The animals actually swim in a spiral path, continuously revolving on their long axes as they go.

Find a relatively quiet specimen and examine with high power. There are many species of Euglena which differ considerably in size and shape. The body is generally elongate, tapering at both ends, spindle-shaped or cigar-shaped. Some species are, however, flattened. Is the shape permanent or changeable as in the Amoeba? Are pseudopodia formed? Does the animal have definite anterior and posterior ends? Specimens of Euglena frequently cease swimming and undergo *euglenoid movements,* consisting of wormlike contractions and expansions. The flat species tend to twist into screwlike shapes.

2. **Detailed structure.**—As in Amoeba, the body is divided into two regions, a surface layer, the *ectoplasm* or *ectosarc,* and an internal mass, the *endoplasm* or *endosarc.* The ectoplasm consists chiefly of a more or less definite cell membrane, called the *cuticle or pellicle.* It is elastic, permitting considerable changes of shape. In some species,

particularly the larger forms, it is obviously striped with diagonal lines, or *striations*, but these are absent or indistinct in other species. Presumably they serve to strengthen the cuticle. The endoplasm of Euglena is more or less filled with green bodies called *chloroplasts*. The green material is *chlorophyll*, the same material that makes plants green. The chlorophyll in green plants also exists in the form of definite bodies, or masses, the chloroplasts. (At this point a bit of green plant may be examined under the microscope; note green chloroplasts in the cells.) Owing to this possession of chlorophyll, Euglena and other green flagellates are frequently regarded as plants. They are able to manufacture carbohydrates as plants do and hence need not take any solid food. Euglena, however, has been observed to feed on bacteria. In some species of Euglena one or more large ring-shaped or squarish bodies may be present in the endoplasm among the chloroplasts. These are called *paramylum bodies* and are composed of a kind of starch manufactured by the chloroplasts and stored in this manner. In other species of Euglena the paramylum usually occurs as small rods or granules.

The *anterior* end of Euglena is somewhat blunt, the *posterior* end usually pointed. At the anterior end is a shallow depression, the *gullet* or *cytopharynx*. The entrance to this is the *mouth*. Near the anterior end is a red granule, the *eye spot* or *chromatophore*, believed to serve for the perception of light. Near the chromatophore is a round clear spot, the *reservoir*, which is said to receive the contents of several minute *contractile vacuoles* (impractical to see) and to discharge them (without contracting) into the gullet.

The *flagellum* of Euglena is often very difficult to see, because its optical properties are much like those of water. It is a long threadlike process, springing from one wall of the gullet. It is in constant motion around the anterior end of the animal. To see it, try changing the light in various ways or sitting in different parts of the room. It is often glimpsed as a shadow-like line as it is whipped about by the animal. The flagellum is readily absorbed and reformed by Euglena and specimens, particularly those undergoing euglenoid movements, frequently lack a flagellum.

Draw a Euglena, showing details of structure.

3. Reproduction and encystment.—Euglena reproduces usually by *longitudinal fission*. This is rarely seen by students. *Encystment* is common and is supposed to be a response to unfavorable conditions. In this process one or two individuals round up and secrete a wall about themselves. This *cyst* wall protects the inclosed individuals from drying and other unfavorable conditions. Within the cyst, fission may occur. When good conditions recur, the animals emerge from the cyst and resume their normal activities. **Draw** a cyst if you find one.

4. General considerations.—Compare Euglena with Amoeba as to definiteness of form and differentiation of structure.

C. PARAMECIUM

Paramecium is the most common representative of a large group of Protozoa known as *ciliates* and distinguished by their possession of *cilia* as locomotive structures.

1. General form and movements.—Mount a solid piece of scum and a drop of water from the culture and examine under low power without a cover glass. A number

of different animals will probably be present; of these the relatively large, greenish, slipper-shaped, swiftly moving, animals are Paramecia. Note carefully the general shape of the body. Are the two ends different in form? Is one end always directed forward in swimming? If so, which end? Does the animal have a definite permanent shape?

Watch and describe in your notes the swimming movement. Observe that the animal when swimming freely revolves upon its long axis and also seems to swerve from side to side. What is the cause of the rotation and swerving and what is the real path of the animal (see texts)?

Observe that the animal is not symmetrical and cylindrical but the anterior half is deeply concave as if a large slice had been cut out of it. This concave depression is called the *oral groove*. It is best seen by watching the animals as they revolve. The side bearing the oral groove is regarded as the *oral* or *ventral* surface; the opposite surface as *aboral* or *dorsal*; *right* and *left* are then defined as in other animals. Watch the freely moving animals carefully until you have obtained a correct idea of the width, length, and direction of slant of the oral groove. How wide is it at the anterior end? How far posteriorly does it extend? Does it slant from right to left or left to right? (Remember that as the animal is transparent you cannot determine whether you are looking at the dorsal or ventral side of it except by the aid of the focusing screw.)

Make an outline drawing of the animal from the *ventral* side, about four inches long, showing its correct shape and proportions and correct appearance of the oral groove. Have the drawing approved before proceeding. The details of structure are later to be entered on this outline.

2. Detailed structure.—This study must be made with the high power and some method of quieting the animals is necessary. One or all of the following methods should be tried. (1) Always mount the animals with a piece of scum or other solid material from the culture. They tend to remain quiet about such objects or around air bubbles or at the edge of the cover glass. This is the most satisfactory method, as the animals remain normal throughout. Every student should try this first. (2) Withdraw water by applying a piece of filter paper to the edge of the cover glass. The animals, not having sufficient water in which to swim, remain motionless but lose their normal shape and appearance and soon burst and die so that observations must be completed quickly. (3) Try mounting in Chondrus jelly. (4) Add a drop of dilute formaldehyde or 4 per cent propyl alcohol to a drop of Paramecium culture and cover. The animals should slow down after a time. See Appendix for further details of these methods.

All observations with the high power must be made with a cover glass on the material. Do not allow the animals to dry up. Study only normal specimens as far as possible.

a) *Ectosarc:* The outer layer of Paramecium is called the *ectoplasm* or *ectosarc* The surface of the ectosarc is differentiated as a firm membrane, the *cuticle* or *pellicle*, to which the animal owes its permanent shape. This cuticle is marked with a mosaic of hexagons but these are not readily seen. From the cuticle arise numerous hairlike processes, the *cilia*, whose co-ordinated movements propel the animal through the water. The cilia are best seen by focusing on the edge of the animal. Are they of the same

length all over the body? Those in the oral groove are especially active. The layer of ectoplasm under the cuticle contains numerous rodlike bodies, the *trichocysts*, lying parallel to each other and at right angles to the surface. To see them, focus on the edge of the animal. They are believed to be little sacs filled with a viscous fluid. **Add** cilia and layer of trichocysts to your outline drawing. Do not make a new drawing.

b) *Endosarc:* The central mass of Paramecium is the *endoplasm* or *endosarc*. It is granular and much more fluid than the ectosarc. It generally contains a number of *food vacuoles*, spherical drops of fluid filled with food particles. The *nuclei* are located in the endoplasm (see below). **Add** details of the endoplasm to your drawing.

c) *Digestive apparatus:* This is best studied on a normal active animal, one that is resting quietly near some object. It can also be seen in formalized or alcoholized animals but *not* on those flattened out by withdrawal of water. Having found a favorable individual, study with high power and watch the posterior end (be sure you know where this is) of the oral groove for an oval clear spot, the *mouth*. Then look for a clear funnel-shaped curved cavity leading posteriorly from the mouth down into the endoplasm. This is the *gullet* or *cytopharynx*. In favorable cases you may observe minute food particles shooting down the gullet and collecting into balls at its inner rounded end. Note the great activity of the cilia in the gullet, where they are fused into a vibrating membrane, known as the *undulating membrane*. The oral groove, mouth, gullet, and undulating membrane are the food-catching apparatus of the Paramecium. Near the gullet is a region called the *anus* where undigested food is extruded. It can be seen only when material is ejected through it. **Enter** these details on your drawing.

d) *Contractile vacuoles:* Observe a circular clear spot near each end of the animal, situated between ectosarc and endosarc. These are the *contractile vacuoles*. Are they fastened in a definite location? Are they on the dorsal or ventral side? Watch the contraction and note the radiating *canals* which appear like the petals of a flower around the place where the vacuole disappeared. What is the average time between successive contractions of a vacuole? Compare with the contraction interval in Amoeba. (The normal behavior of the vacuoles can be observed only on active specimens as the vacuoles slow down when the animals are narcotized and become large and abnormal in flattened specimens.) **Add** vacuoles and canals to your drawing.

e) *Nuclei:* Ciliate Protozoa have two kinds of nuclei, a large *meganucleus* (also called *macronucleus*) and one to many small *micronuclei*. They cannot be seen in normal animals but the meganucleus is often dimly visible as a central denser mass in flattened specimens. Study the nuclei on stained slides provided in your set or add aceto-carmine stain to living specimens. The appearance of the nuclei differs in different species of Paramecium. The meganucleus is a large irregular mass in or near the center. In some species of Paramecium, the micronucleus is a small round body lying in a depression of the meganucleus; in other species there are two to several micronuclei, very difficult to see. **Draw in nuclei.**

3. Experiments on Paramecium.—

a) *Formation and course of food vacuoles:* Start this experiment at the beginning of the laboratory period, as it requires some time for completion. It need not be watched continuously and the other experiments may be performed during its progress. Mount

some Paramecia with a piece of scum, add a drop of carbon suspension, and cover. Find an individual resting about the piece of scum or elsewhere. Note that the particles of carbon are swept down the gullet to its rounded termination where they collect into whirling spherical masses. Note from time to time passage of these masses into the endoplasm as a food vacuole. Observe in what part of the body the food vacuoles first collect and where they occur later. Examine at intervals (do not let the preparation become dry) and determine the exact course of the food vacuoles in the endoplasm. This movement is due to a slow circulation of the endoplasm. If you watch long enough, you may see discharge of the carbon through the anus. **Make a diagram** showing course of food vacuoles in the endoplasm.

The experiment may also be performed using instead of carbon suspension, bacterial scum stained with neutral red. Neutral red is red in neutral, purple red in acid, and yellow in alkaline solution. By the color of the food vacuoles determine any changes in acidity or alkalinity during the progress of digestion.

b) *Discharge of the trichocysts:* Mount some Paramecia on a slide, add a drop of picro-acetic acid, cover gently, and examine. Each animal will be found surrounded by a halo of long threads. These threads are formed by the discharge of the contents of the trichocysts through minute holes; the contents are thus squeezed into the form of a thread and solidify on contact with water. The function of these threads is not known. **Make a drawing** showing relative lengths of cilia and trichocyst threads.

c) *Avoiding reaction:* Mount some Paramecia and observe under low power. What happens when one of them strikes an obstacle? This reaction is called the *avoiding reaction*. **Make a diagram** to illustrate the reaction, showing the position of the Paramecium before, during, and after striking the object.

d) *Reaction to chemicals:* Clean a slide thoroughly. Obtain a large number of Paramecia and spread them out over the whole slide. Lay the slide on the table and drop a minute crystal of common salt in the center of the slide. Observe with the naked eye or a hand lens the behavior of the animals toward the salt. What does each Paramecium do on coming into the vicinity of the salt? What part does the avoiding reaction play in the behavior? **Make a diagram** to show distribution of Paramecium with regard to the salt, indicating the animals by dashes. The reaction to other chemicals may be tested in the same way.

4. Reproduction.—Reproduction is accomplished by the fission (division) of the animal into two halves by a transverse constriction. A sort of sexual act called *conjugation* also occurs at intervals but is not necessary for the continued existence of Paramecium.

a) *Fission:* Both nuclei take part in fission, the meganucleus dividing by direct division, the micronuclei by a kind of mitosis. **Study and draw** from prepared slides three stages of fission—early, middle, and late. Each slide bears only a few individuals in fission, their location being generally indicated by marks on the cover glass. Be sure you have a specimen in fission before drawing.

(1) *Early fission:* The beginning of fission is recognizable by the great elongation of the meganucleus. The micronucleus (if more than one, all behave alike) moves out into the general cytoplasm and having formed spindle fibers and chromosomes

within itself pulls into two halves which move farther and farther apart drawing out the connection between them into a thinner and thinner thread which finally ruptures. Specimens in early fission should show some stage of this process.

(2) *Middle fission:* The meganucleus is separating into two halves connected by a slender thread. A transverse constriction is present across the center of the cell. The micronuclear division is completed and one or more micronuclei are present in each half.

(3) *Late fission:* The meganuclear division is completed and the cytoplasmic constriction has divided the cell nearly or completely in two, though both halves cling together.

b) *Conjugation:* Look over the slide for cases in which two Paramecia are united by their oral grooves. **Draw.** Consult your textbook on the nuclear changes in conjugation.

5. General considerations.—Is Paramecium more differentiated than Amoeba? In your answer compare Amoeba and Paramecium in detail as to definiteness of form, organs of locomotion, place of ingestion and egestion of food, etc. In what part of the protoplasm, ectoplasm, or endoplasm, has the greater degree of differentiation occurred? Can you give a reason for this? Do you think Paramecium gains anything by its more complex structure? Compare behavior of the two animals. Has Paramecium a greater variety of reactions than Amoeba? Is this of advantage?

D. GENERAL STUDY OF PROTOZOAN CULTURES

To obtain Protozoa in numbers they must be provided with an abundance of food. This is done by making a *culture,* which consists of a quantity of water containing boiled hay, grain, bread, pond weeds, or other food materials. The culture is seeded with Protozoa by adding a little water or débris from a pond. Bacteria and other fungi are nourished by the food in the culture and in turn serve as food for Protozoa and other small animals.

Obtain a drop or two of water from a Protozoan culture and examine. In addition to bacteria, yeasts, molds, etc., the drop will contain animals of two kinds: one-celled animals or Protozoa; and multicellular animals, or Metazoa.

1. Protozoa.—Learn to distinguish Protozoa from other microscopic animals. They are recognizable by absence of cell walls and organs, and structureless granular appearance of their bodies. Study several kinds found and pay particular attention to organs of locomotion and other special structures. Among the common Protozoa found in cultures are the following. **Drawings** are desirable.

a) *Vorticella:* This ciliate protozoan consists of a bell-shaped body situated on the end of a slender stalk which is attached to some object. Note contractions and expansions of bell and stalk at intervals. The stalk contains a spiral *muscle* which on shortening draws the stalk into the form of a spring. We thus see that muscle fibrils can be differentiated within the limits of a single cell. The free end of the bell bears a circlet of large cilia and an opening into the gullet. Look for food and contractile vacuoles. Stain with aceto-carmine to see the nucleus.

b) *Stentor:* This ciliate has a trumpet-like form and is usually attached to objects.

It may swim free, whereupon it assumes an oval form. Note circlet of large cilia around the broad end, conspicuous spiral gullet, contractile vacuole, muscle stripes in the ectoplasm, and meganucleus resembling a string of beads.

c) *Hypotrichous ciliates:* This group of ciliates is easily distinguished by a jerky darting method of locomotion and the possession of large bristle-like cilia which are used as legs in creeping. These bristles, known as *cirri*, are really fused bundles of cilia. Look for gullet, contractile vacuole, etc.; stain with aceto-carmine to see the meganucleus. Common forms are *Euplotes, Stylonichia, Oxytricha.*

d) *Other ciliates:* Ciliates are the commonest Protozoa and a great variety may be expected in cultures. Some forms frequently seen are: *Frontonia*, similar to Paramecium but more oval in form; *Didinium*, oval with a short proboscis at the anterior end and two girdles of cilia encircling the body; *Spirostomum*, long, slender, cigar-shaped with oblique muscle stripes; *Lacrymaria*, spindle-shaped with a very long slender mobile "neck"; *Calceolus*, body constricted in the middle, one girdle of cilia, with a tail-like posterior process; *Colpoda* and *Colpidium*, small oval forms with an indentation near the anterior end; *Coleps*, with an armor of squarish plates; *Halteria*, a very small spherical form, moving by swift darts; *Dileptus*, a large form with a short contractile "neck"; *Loxodes*, moderate size, elongate, flattened, anterior end curved to left, hooklike.

e) *Heliozoa or sun animalcules:* These are spherical protozoans with stiff radiating pseudopodia and bubbly protoplasm. They are related to Amoeba. *Actinophrys*, small, and *Actinosphaerium*, quite large, are the common kinds.

f) *Amoeboid organisms:* The only other common protozoan, which is similar to Amoeba, is *Arcella.* This animal resembles Amoeba in all respects except that it is inclosed in a brown hemispherical shell.

g) *Flagellates:* Protozoa similar to Euglena are not uncommon in cultures. Some are green, some brown, most of those found in ordinary cultures are colorless, with one or two flagella.

2. Metazoa.—Metazoa which occur in cultures are known by the presence in their bodies of definite organs. The following are common in cultures. See texts for the characters of the different phyla.

a) *Rotifers or wheel animalcules* (Phylum *Trochelminthes*): These extremely common animals are known at once by the presence at their anterior ends of one or two disks of cilia, whose constant beating produces the illusion of rotating wheels. The ciliary disks can be folded into the head. Other interesting features are the internal jaws which exhibit a constant chewing movement, the jointed posterior end often provided with one or more "toes" for clinging, and the hard case or *lorica* which often incloses the animal. The most common rotifer in cultures is *Philodina*, which possesses a pair of "wheels."

b) *Roundworms or nematodes* (Phylum *Nemathelminthes*), slender cylindrical, wormlike animals, pointed at each end, without cilia, moving by violent alternate curving and straightening of the body.

c) *Flatworms* (Phylum *Platyhelminthes*), slender, flattened animals, moving in a smooth gliding manner, due to the presence of cilia; with more or less definite heads.

Stenostomum is the most common form and often occurs in chains, produced by fission.

d) *Chaetonotus* (Phylum *Trochelminthes?*): This animal, possibly related to the rotifers, resembles a ciliate protozoan. The whole body is covered with short spines; is slender and flexible, with a rounded head region, a short "neck," and two pointed processes at the posterior end.

e) *Naids* (Phylum *Annelida*), slender, cylindrical, mostly rather large, worms, provided with projecting bristles at intervals. The smallest ones belong to the genus *Aeolosoma* and have red, green, or orange spots. Some (*Stylaria, Pristina*) have a long slender, active proboscis at the anterior end. *Chaetogaster* is very transparent. *Dero* is provided at the posterior end with an expansible hood bearing several ciliated gills.

f) *Water mites and water bears* (Phylum *Arthropoda*): These are round to oval flat animals with projecting legs. The water mites have six jointed legs when young, eight when adult. The water bear has eight short non-jointed legs.

g) *Entomostraca* (Phylum *Arthropoda*): These common animals have many pairs of conspicuous jointed legs and often large jointed antennae projecting from the head. They swim in a jerky manner. One of the commonest types is *Cyclops*, a small animal with a jointed body, single eye, long swimming antennae, and posterior terminal spines; females often with two sacs full of eggs hanging to the body. The *daphnids* or *water fleas* have well-marked heads with large eyes, and powerful swimming antennae, bodies inclosed in a shell through which the jointed legs are visible. The *ostracods* are a group of Entomostraca completely inclosed in a bivalve shell from which the legs protrude at intervals.

h) *Insect larvae* (Phylum *Arthropoda*): Insect larvae look like worms from which they may be distinguished by the presence of definite brown hardened heads. They have elongated jointed bodies often provided with tufts of bristles, and may or may not have three pairs of jointed legs.

IX. PHYLUM COELENTERATA

The phylum Coelenterata includes an assemblage of animals distinguished by their *diploblastic* structure, *radial* type of symmetry, and possession of *tentacles* armed with stinging bodies (*nematocysts*). They are nearly all marine but there is one member of this phylum, a little animal named *Hydra,* which is common in fresh waters.

A. HYDRA

1. General structure.—Examine the culture jar containing Hydra and note general appearance and size of the animals. They are *sessile,* that is attached to the glass, plants, etc. The free end of the body is surrounded by a circlet of threadlike processes, the *tentacles,* which extend free into the water. Do the animals show any distribution in the jar with regard to light or air? Do they exhibit movements? Describe in your notes. There are a number of species of Hydra, of which only the green Hydra, *Hydra viridissima,* and the brown Hydra, *Hydra oligactis,* with stalked body and very long tentacles, are easily recognized.

Obtain a living Hydra in a watch glass with sufficient water to cover the animal. Handle it carefully. Examine with the lowest power of the microscope or with a hand lens.

The body or *column* of the animal is a cylindrical elastic tube, capable of great extension and contraction. In Hydra oligactis the anterior half of the column is much stouter than the basal half, which is reduced to a slender *stalk;* in the other species, the column is of about the same diameter throughout. From the free end of the column, called the *oral, anterior,* or *distal* end, arises a circlet of *tentacles,* variable in number, usually five or six being present. The oral end between the bases of the tentacles forms a conical elevation, the *hypostome,* in the center of which the *mouth* is situated. The mouth when closed is star-shaped but is usually difficult to see unless the hypostome faces directly upward. The attached end of the animal is called the *posterior, aboral,* or *proximal* end and terminates in a disk, the *foot* or *basal disk,* which secretes a cement-like substance serving to attach the animal. Column and tentacles are hollow, containing a cavity known as the *gastrovascular cavity,* so called on the supposition that it has circulatory as well as digestive functions.

Examine the tentacles and note that they bear numerous protuberances each of which contains a collection of very small sharply outlined bodies, the *stinging cells* or *nematocysts.* They also occur sparingly on the column.

Draw a Hydra in the extended state showing general features.

In the first chapter of this book the matter of symmetry in animals was discussed. Reread this. Then compare the symmetry of Hydra with that of the frog or man. Hydra is not bilaterally symmetrical as the latter are but is *radially symmetrical,* that is its parts radiate from a central axis, the *apico-basal* or *oral-aboral axis,* so that a number of planes could be passed through this axis which would divide the animal into similar

halves. Does Hydra have any right or left sides, dorsal or ventral surfaces? Is the anterior end of Hydra more differentiated than that of Paramecium and other Protozoa? than that of the frog or man?

2. General behavior.—On a specimen in a watch glass or on those in the culture jar perform the following experiments. Use a clean needle, touch the animals gently, and wait for the animal to expand completely before stimulating it again or use a different specimen. Touch one tentacle of a fully expanded individual gently. Does it contract? Do other parts contract? Touch two or three tentacles simultaneously. Is the response more marked than before? Try the comparative effect of a touch of the same intensity on the foot, middle of the column, and hypostome. What parts of the animal are the most sensitive? least sensitive? Compare reaction time with that in the experiments in chapter ii. Try difference in response to a weak and a strong touch applied to the same region. Jar the water of the general culture and note result. Do any of these experiments indicate that the stimulus is conducted from the point of stimulation to other parts? Hydra, like the frog and man, has sensory cells at the surface which perceive stimuli, a nervous system which conducts them, and muscle fibers which respond by movement.

Make an outline drawing of a fully contracted Hydra.

Note or mark positions of several Hydras in the general culture. Examine every day and determine if the animals move about. Observe, if possible, methods of locomotion. See texts.

Add some small Crustacea to the Hydra culture and observe, as carefully as you can, capture and swallowing of the prey by Hydra. Into what part of the column is the prey taken? Observe attitude and state of expansion of column and tentacles of individuals that have fed compared with those that have not. Note pale bloated appearance during digestion. About how long is the prey retained in the gastrovascular cavity? Is the process of digestion in Hydra like that of man or frog or like that of Protozoa or like both? See texts.

3. Cellular structure of the living animal.—Mount a small Hydra on a slide. Cut some small slivers of wood of the right thickness to prevent the cover glass from crushing the animal, yet permitting focus with the high power. Place the slivers around the Hydra, put the cover glass gently on the slivers, and fill up the space under the cover with water. Examine with high power. The animal must be fully extended for the following observations.

a) *Cellular structure of the column:* Examine column near the foot. First focus on the surface and note that it consists of a mosaic of polygonal cells, the *ectoderm* cells. Then focus slowly downward and observe that a layer of larger clear cells, the *entoderm* cells, comes into view. In the green Hydra these two layers are very distinct, as the ectoderm is colorless, the entoderm green. The two layers can be still better seen by obtaining an *optical focus* on the edge of the animal. (This means to focus on the center so that the appearance is the same if you had made a section through the animal.) In such a focus the wall of the animal is seen to be composed of an outer thin *ectoderm* layer, an inner thicker clear *entoderm* layer, and a dark line, the *mesogloea,* between the two layers. Note also the central *gastrovascular* cavity. This study shows

us that Hydra is a *diploblastic* animal, that is, is composed of two layers, an outer and an inner, like two cylinders one within the other. Small sharply outlined oval bodies scattered over the column are *nematocysts.*

b) *Cellular structure of the tentacle:* Examine the base of a fully extended tentacle and repeating the foregoing procedure, note ectoderm cells on the surface, large clear entoderm cells in the interior on focusing downward, and mesogloea between them. The center of the tentacle is occupied by a slender canal, a branch of the gastrovascular cavity, usually not very distinct. Examine the more distal portions (those farther away from the body) of the tentacles and note the arrangement of the stinging "cells" or nematocysts. They occur in groups, known as *batteries,* which cause the conical projections readily noticed in profile view of the ectoderm. Examine a battery more closely. It is inclosed in a single ectoderm cell, is made up of large and small nematocysts more or less definitely arranged, and bears projecting spines, the *cnidocils.* Draw a small portion of the tentacle to show cell structure and batteries.

4. The nematocysts.—Remove the supports from under the cover glass, letting the latter rest directly on the Hydra, and drum on the cover glass with the point of a pencil until the animal is thoroughly crushed. This frees the nematocysts and causes some of them to discharge. Examine with high power. There are always four kinds of nematocysts, a large barbed and three smaller varieties. Each frequently is inclosed in a cell, the *cnidoblast,* which forms it and which bears the cnidocil.

a) *Large barbed nematocysts or penetrants:* In the undischarged state these are oval sacs with a flat end from which a pouch bearing a coiled thread projects into the interior of the sac. When discharged, pouch and thread are ejected to the exterior, turning inside out. The pouch is then seen to bear three large spikes, several smaller ones, and a long hollow thread.

b) *Small oval nematocysts or glutinants:* These vary in different species from oval to pear-shaped and are of two kinds, one larger than the other. The thread is variously coiled inside and fine and long on discharge.

c) *Small spherical nematocysts or volvents:* These are a little smaller than the preceding, more spherical in form, and contain, when undischarged, a short thick thread curved in a single loop. When discharged this thread makes a tight little coil of three or four turns.

Draw as many kinds of discharged and undischarged nematocysts as you can find without spending too much time on them.

5. Cellular structure from slides.—Examine a cross-section through Hydra with the low power. Note that the section consists of two circles of cells, an outer *ectoderm* and an inner *entoderm,* surrounding a central gastrovascular cavity. The section clearly shows the diploblastic structure of Hydra. Turn on the high power and study carefully the kinds of cells present.

a) *Ectoderm:* The ectoderm is composed mainly of medium-sized cells, known as *epithelio-muscular* cells, whose boundaries are frequently indistinct. Each such cell consists of a polyhedral *epithelial* portion which takes part in forming the ectoderm and a basal portion which is drawn out into a long slender *muscle fibril* running in a longitudinal direction. Thus the bases of all of the ectoderm cells of Hydra form a

longitudinal muscle coat for the animal. (This muscle coat is situated in the mesogloea, see below.) Each epithelio-muscular cell has a large nucleus, containing a nucleolus and a distinct network. Within many of the ectoderm cells, nematocysts, usually somewhat distorted, will be noticed. Inside of or between the bases of the epithelio-muscular cells occur here and there groups of small dark granular cells, each about the size of a nucleus of the epithelio-muscular cells. These are the *interstitial* cells, from which nematocysts and sex cells originate.

b) *Entoderm:* The entoderm is made up chiefly of the *nutritive muscular* cells, large, elongated, vacuolated cells, with well-defined cell membranes, and bulging crowded free ends (ends next the cavity). They usually contain many food particles and droplets, have a nucleus like those of the ectoderm cells, and a small amount of granular protoplasm in the free end, the remainder of the cell consisting mostly of a large fluid-filled space, or vacuole. As in the case of the ectoderm cells, the bases (attached ends) of the entoderm cells are drawn out into muscular fibers which run in a *circular* direction, forming a *circular muscle coat*, which is, however, much more poorly developed than the longitudinal coat. Situated between the free ends of the nutritive muscular cells is another type of entoderm cell, the *secretory* or *gland* cell, believed but not proved to produce digestive enzymes. These gland cells are small triangular cells filled with granules or with a network left by the dissolution of the granules.

c) *Mesogloea:* The mesogloea is not a cell layer but is a sheet of gelatinous material cementing the ectoderm and entoderm together. It contains the muscle fibrils of both ectoderm and entoderm but only the former are distinct and as they are cut crosswise in cross-sections they appear as dark dots imbedded in the mesogloea.

Draw a small portion of the section in great detail to show all of the kinds of cells and structures present.

6. **Reproduction.**—Hydra reproduces by the asexual method of budding or sexually.

a) *Budding:* Examine living specimens or whole mounts showing budding. A bud begins as a rounded projection of the body wall, consisting of all layers. It elongates and at its distal end, little knobs which become the tentacles appear. It develops into a small Hydra which then separates from the parent. A Hydra may have one to several buds. Do they occur at a definite region of the column? **Make a simple outline drawing.**

b) *Sexual reproduction:* Hydra generally develops sex organs only at a certain time of the year, the time varying with the species. Some species of Hydra are hermaphroditic and others are strictly dioecious. Examine living specimens or whole mounts.

(1) *Male reproductive organs:* The male organs, *spermaries* or *testes*, consist of conical elevations in the ectoderm, with or without a nipple-like process for the exit of the sperm. They are distal in hermaphroditic, but extend to the budding zone in dioecious species. **Draw the specimen.**

(2) *Female reproductive organs:* The *ovaries* are low broad elevations, without a nipple, containing when ripe an amoeboid egg, which finally ruptures through the ectoderm. **Draw the specimen.**

7. General considerations on Hydra.—Hydra shows an advance over Protozoa in that it consists of several different kinds of cells, each with specific functions, and arranged in definite layers, which foreshadow the systems and organs of the higher animals. We may notice further that Hydra is not merely a collection of several different kinds of cells but its cells act together for the general welfare of the animal. Each one is more or less helpless without the others since it has become specialized for particular functions and hence cannot successfully perform all functions like a protozoan cell. Hydra is, therefore, an *individual,* a unity produced by co-operation, ruled by a *dominant* region, the *anterior end* or *head,* which, to be sure, is not yet very distinctly differentiated from the rest of the body.

To what stage of embryonic development does a Hydra correspond? Which of its two layers is more differentiated and what reason can you give for this? In Hydra, integumentary, digestive, muscular, reproductive, nervous, and sensory systems are present in very simple condition. What other systems present in complex animals (see p. 9) are lacking in Hydra and how does it accomplish the functions performed by these systems when they are present?

B. A COLONIAL COELENTERATE

These animals, relatives of Hydra, are common inhabitants of the shallow waters of the ocean. Preserved material of either *Obelia* or *Campanularia* may be used. Obtain a preserved colony and examine in a watch glass of water.

1. General structure.—The colony has a branching plantlike appearance. Identify the rootlike basal branches, or *hydrorhiza,* by which the colony is attached to solid objects, the main stem, or *hydrocaulus,* and the *branches,* which bear enlarged rounded objects, each of which is an individual similar to Hydra and is called a *zooid, hydranth,* or *polyp.* The colony is spoken of as a *hydroid colony.* The zooids are produced by budding from the stems as in Hydra. In fact, if the buds of Hydra should remain attached to the parent a colony similar to a hydroid colony would result. **Make a small sketch** of the colony to show its general appearance.

2. Detailed structure.—Mount some of the preserved material on a slide or use the prepared slide in your set. Examine with low power.

a) *Stem:* The stems are covered with a brown horny layer, the *perisarc.* Within the perisarc is the *coenosarc,* a hollow tube comparable to the column of Hydra and consisting, like the latter, of ectoderm (which secretes the perisarc), mesogloea, and entoderm. Its cavity, the gastrovascular cavity, is continuous throughout the colony. The perisarc is ringed at the base of each branch, probably to make the stem more flexible.

b) *Nutritive zooids:* There are two kinds of zooids of which the more abundant kind is called the *nutritive zooid.* It is similar to the distal part of a Hydra. It consists of an elongated *body* from which arises the club-shaped *manubrium,* terminating in a wide *mouth* opening. At the base of the manubrium is a circlet of *tentacles* bearing *nematocysts.* The perisarc extends around each nutritive zooid in the shape of a wine-glass, the *hydrotheca.* **Draw a nutritive zooid.**

c) *Gonozooids:* These modified zooids occur in the angles between the hydrocaulus and the branches. Each is inclosed in a cylindrical case of perisarc, the *gonotheca,* open at the free end. The gonozooid consists of a central stalk or *blastostyle,* which is a degenerate zooid, and a number of saucer-shaped bodies, the *medusa buds,* borne on the blastostyle and almost concealing it from view. The medusa buds are formed by a process of budding from the blastostyle and usually escape eventually (not in Campanularia) as little bell-shaped animals, called *medusae,* which swim about free in the ocean. **Draw** a gonozooid.

3. **Medusae.**—The medusae of Obelia are very small and should be examined as whole mounts. Preserved specimens of larger hydroid medusae may also be used. Note bell-shaped or saucer-shaped gelatinous body, called the *bell.* Its jelly-like composition is due to an enormous development of mesogloea. The margin of the bell bears *tentacles* armed with *nematocysts.* From the center of the concave surface of the bell depends a short or an elongated structure, the *manubrium,* at the free end of which the *mouth* is located. From the attached end of the manubrium, *radial canals,* usually four in number, radiate to the periphery of the bell where they join a *circular canal,* running around the margin. These canals constitute a food-distributing system; their cavities form the gastrovascular cavity of the medusa. The reproductive *organs* generally occur along the under surface of the radial canals. A circular muscle shelf, the *velum,* extends inward from the margin of the bell and by its contractions, enables the animal to swim about. The medusae are the sexual individuals of the hydroids. They give rise to eggs and sperm (usually on separate medusae) and the fertilized egg develops into a new hydroid colony. **Draw.**

4. **General considerations.**—In these colonial coelenterates, there exist then three kinds of individuals: the nutritive zooid, whose function is that of food getting and digestion; the gonozooid whose function is to bud off medusae; and the medusa, whose function is reproduction. Such a condition is known as *polymorphism* or *division of labor,* because the organism instead of differentiating into organs for the performance of different functions (a procedure which its simple diploblastic structure does not, indeed, permit) differentiates into several kinds of individuals for the performance of various functions. The hydroids also illustrate the principle of *metagenesis,* or *alternation of generations,* that is, the medusa, or sexual generation, is thought of as alternating with the hydroid colony, or asexual generation. It would probably be advisable to drop this idea altogether and regard the medusa simply as the final or adult stage in the development of the animal.

C. GENERAL SURVEY OF OTHER COELENTERATES

Study the collection of coelenterates provided, reading what your text has to say about them. Acquaint yourself with the general appearance of the following common groups of coelenterates.

1. **Hydroids.**—These animals are extremely common along the shores of the ocean, forming beautiful plantlike growths on rocks, wharves, plants, shells of animals, etc. Note various types of branching, size and shape of zooids, etc.

2. **Medusae.**—Medusae, as stated above, are the sexual stage of hydroid colonies.

They are of various sizes and vary in shape from flat saucer to tall bell shapes. Their structure is similar to the medusa of Obelia described above.

3. True jellyfishes.—These differ from medusae, which are also often called jellyfishes, in the absence of a velum, larger size, and more complicated structure. They may or may not originate from a hydroid stage.

4. Siphonophora.—These strange free-floating hydroid colonies illustrate the principle of division of labor carried to its highest development, since they may consist of seven or eight different types of individuals. The well-known *Physalia* or *Portuguese man-of-war* has a large "float" (which is a modified medusa) from the lower side of which the other members of the colony hang down with long trailing tentacles. The *Vellela* or *purple sail* has a flattened disklike float bearing an erect "sail" with zooids on the under surface of the float. In other types of Siphonophora in place of the float there may be a circle or chain of swimming bells which are modified medusae.

5. Sea anemones.—These beautiful animals of shallow marine waters resemble an enlarged hydroid polyp, but their internal structure is considerably more complicated and they have no medusa stage. They consist of a stout column topped by an oral disk covered with tentacles.

6. Stony corals.—These are the common corals which build up reefs and keys. They are small animals of the same structure as sea anemones. They secrete external skeletons of limestone which persist after they perish, forming coral rock. In all dry specimens of coral the animals are, of course, missing, but the cuplike depressions which they occupy in life are readily recognizable. When the cups are incomplete laterally, so as to be fused into long furrows, the type of coral known as "brain" coral results.

7. The horny corals and their relatives include the coral used in jewelry, the organ-pipe coral, and other forms. The animals which secrete these skeletons have eight feathery tentacles.

8. The sea feathers, sea fans, etc.—These plantlike horny skeletons are built up by minute zooids, which occupy in life the small holes which will be found on closer examination distributed abundantly over the skeletons.

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X. PHYLUM PLATYHELMINTHES

The flatworms constitute a phylum of the animal kingdom characterized by pronounced bilateral symmetry, triploblastic structure, absence of a coelome and an anus, and a characteristic excretory system provided with flame-cells. Many of the members of this phylum are parasitic; those which are free-living inhabit salt or fresh waters or in a few cases damp earth. Flatworms of the genus Planaria and related genera are among the common animals of ponds and streams throughout the United States.

A. A PLANARIAN WORM

1. General appearance and behavior.—Obtain a living planarian in a watch glass and examine with a hand lens. Compare form, symmetry, and arrangement of parts with those of Hydra and the frog (or man). What type of symmetry does the animal possess? Does it have anterior and posterior ends? Dorsal and ventral surfaces? Right and left sides? Is the head better differentiated than in Hydra? Note color and differences in color between dorsal and ventral surfaces.

The anterior end of the animal is more or less marked off as a *head.* In many planarians the head has the shape of an arrow head; in others the anterior margin is more or less straight. On the dorsal surface of the head is placed a pair of *eyes,* or in some species, several eyes on each side. Each side of the head bears a projecting lobe or *auricle.*

Observe the peculiar gliding form of locomotion. To what is it due (*A*)? Does the animal exhibit muscular movements also? How do the anterior end and auricles behave during locomotion? Does this behavior suggest their function? Touch various parts of the animal with a blunt instrument and note response and relative sensitivity of different regions. Do these responses suggest a better developed neuro-muscular system than in Hydra? Is the planarian able to respond to stimuli more effectively and in a greater variety of ways than Hydra? Note trail of mucus left by the animal as it crawls about.

Make an accurate outline drawing of the animal several inches long.

2. Detailed structure.—Place a small planarian worm on a slide, anesthetize with a few grains of chloretone (*A*), turn it ventral side up and cover with a cover glass, pressing the latter gently so as to flatten the animal. Examine with low power.

a) General regions and surface of the body: The narrow clear border of the animal is the *ectoderm;* the central branching material, generally brown or gray in color, is the *entoderm;* and the lighter material between these, the *mesoderm.* We thus see that the animal is *triploblastic* in structure. Focus on the surface of the body with high power and note the numerous *pigment granules* often arranged in patterns which give the animal its color (some species are colorless and lack pigment granules). Focus on the ectoderm with high power and observe the numerous rodlike bodies, the *rhabdites,* which it contains. The rhabdites when discharged on the surface soften to produce mucus. Examine the edge of the ectoderm at various places—the margin of the head

is a favorable place—for *cilia*. The ectoderm of planarians is ciliated, usually on the ventral surface only.

b) *Sense organs of the head:* Examine an eye. It consists of a clear area, where the sensory cells are located, and a crescent-shaped mass of very black, densely packed granules, whose function apparently is to concentrate the light upon the sensory cells by preventing reflection and diffusion. As the eye of planarians has no lens it cannot perceive objects but can only distinguish different intensities of light. The auricles and margins of the head are important sensory areas containing a variety of sensory cells, principally of touch and chemical sense.

c) *Digestive system:* In the center of the body is an oval clear region, the *pharyngeal chamber,* in which is situated a cylindrical contractile tube, the *pharynx*. The pharyngeal chamber opens on the ventral surface by the *mouth,* which appears as a small circular clear area in the median line near or at the posterior end of the pharyngeal chamber. The pharynx is attached at its anterior end to the anterior wall of the pharyngeal chamber; the rest of it lies free in the chamber and terminates posteriorly in an opening, the entrance to the pharynx, which should not be confused with the mouth. When the animal feeds the elastic pharynx elongates and is protruded through the mouth as a *proboscis*.

At its anterior end the pharynx opens into the *intestine* which divides at once into three main trunks, a median *anterior* trunk which runs forward about to the eyes, and two *posterior* trunks which pass backward, one at each side of the pharyngeal chamber, to the posterior end. Each trunk gives off numerous side branches or *diverticula,* often very irregular in form and often uniting to form accessory longitudinal trunks. The intestine generally appears gray or brown and is easily seen if the animal is well pressed out. Whole mounts of specimens fed on blood or other colored material are useful for the study of the digestive tract.

Add digestive tract to your outline drawing.

The intestine of Planaria differs from the true intestine of the higher animals in that it consists of but a single layer of cells, the entoderm, and is not separated from other structures as a definite tube. Its wall corresponds to the entoderm layer and its cavity to the gastrovascular cavity of Hydra.

d) *Nervous system:* The nervous system is well developed in planarians and can usually be seen in whole mounts. The *brain* or *cerebral ganglia* appears as a pale area around each eye. From these, nerves, seen in whole mounts as pale streaks, pass to the auricles and margins of the head and from each cerebral ganglion a *ventral nerve cord* extends posteriorly on a line with the posterior trunks of the intestine. **Add to your drawing** any part of the nervous system seen.

e) *Excretory system:* This is well developed but very difficult to see. Texts should be consulted.

f) *Reproductive system:* Planarians are hermaphroditic with complicated reproductive systems, unsuitable for beginners to study. See figures in texts.

3. Histological structure.—Examine sections through a planarian worm. The slide bears sections taken through, in front of, and behind the pharynx. Study all three regions. Identify dorsal and ventral surfaces; the former is usually convex, the latter

flat or concave. How can sections in front of and behind the pharynx be distinguished?

Planarians differ histologically from Hydra in that a third layer, the *mesoderm,* composed chiefly of muscles and connective tissue, is present between the ectoderm and entoderm. Their structure is thus triploblastic.

a) *Ectoderm:* This forms a thin layer, the *epidermis,* the outermost layer of each section, composed of cuboidal epithelial cells. It contains rodlike deeply stained bodies, the *rhabdites*. The ventral ectoderm is generally *ciliated,* in some species the dorsal ectoderm also. At each margin is a small area of ectoderm of peculiar appearance, often brightly stained; this area is the place of opening of certain glands, called the *adhesive glands*.

b) *Entoderm:* Each section contains several circular hollow masses which are cross-sections of the intestine. The wall of the intestine is composed of a single layer of tall columnar epithelial cells, often containing *food vacuoles*. These cells constitute the entoderm.

c) *Mesoderm:* All the tissue, except nervous tissue, between the epidermis and the intestine is mesoderm. It is composed of several kinds of tissue. Just under the epidermis is a thin layer of *circular muscles* and internal to this a rather indefinite layer of *longitudinal muscles,* appearing as circles. There are also present *dorso-ventral muscles* extending between the diverticula of the intestine. Numerous one-celled *glands* occur in the mesoderm but are not very distinct unless special stains are used. Of them, the *adhesive* glands in the lateral regions are the most noticeable. The remainder of the mesoderm consists of a kind of connective tissue, called *parenchyma,* composed of branching cells and filling up all space between the other tissues.

d) *The pharynx:* Examine a section through the pharynx. This organ is seen as a circular body lying in a cavity, the *pharyngeal chamber*. In some sections the ventral opening of this chamber, the *mouth,* may be present. As the pharyngeal chamber is a depression of the body surface and the pharynx an outgrowth of the surface, the chamber is lined by and the pharynx covered and lined by ectoderm. The remainder of the pharynx is mesodermal, consisting of circular, longitudinal, and radiating muscle fibers, with parenchyma and gland cells between them.

e) *Ventral nerve cords:* These can usually be recognized (best on sections anterior to the pharynx) as pale round areas, near the ventral surface, about one-third to nearly one-half the distance from the median line to the margin. The pale portion consists of nerve fibers; often a few nerve-cell bodies, with large nuclei, occur along the dorsal border of the nerve cord.

Make a diagram of a cross-section of the worm through the pharynx. **Draw in detail** a small part of the section showing appearance of ectoderm, entoderm and mesoderm.

4. Experiments on behavior.—

a) *Feeding:* Place a small piece of raw meat or a small crushed animal in a dish with planarians that have not been fed for at least a week. Do the worms recognize the presence of food? How? Watch and describe reaction to the food. After the animals have attached to the food, gently lift one up and observe quickly the white protruded pharynx inserted into the food.

b) *Phototaxis:* Place some planarians in a dish and set in the light. Do they go

toward or away from the light? Stir up a dish of planarians so that they are equally distributed throughout and cover one half of the dish with something dark. Observe after a time whether the worms collect in the light or dark half. Repeat on worms from which the heads have been cut off.

c) *Thigmotaxis:* In a dish of planarians that has not been disturbed for some time note whether the animals are aggregated or not. Place a small stone or other object in the dish with worms and observe later if they show any response to it.

d) *Chemotaxis:* Clean a glass plate thoroughly, lay it on the table, and place a worm on it with plenty of water. Put a small grain of salt or a small drop of chemical such as an acid or base in the path of the worm and observe its behavior.

Write up the foregoing experiments fully in your notebook.

5. Regeneration.—Members of each table may work together on the following experiment. Obtain several finger bowls and glass covers for same and clean them thoroughly. Take several planarians and cut them up into pieces of various shapes and sizes. Cut some very small pieces. The animals are best cut by placing them on a glass plate, waiting until they extend to full length, and then making cuts with a quick firm stroke of a sharp scalpel. Place the pieces in the finger bowls with plenty of water, cover with glass plates, keep out of the sun, and change the water every few days. Examine at each laboratory period and make a series of simple outline drawings to show progress of the regeneration.

Is any piece of a planarian worm capable of regenerating a whole worm? Are any differences apparent due to differences in size or shape of the original pieces or part of the body from which they came? Do very small pieces regenerate as completely as larger ones?

6. General considerations on flatworms.—What systems do planarians possess in common with Hydra? Are these better developed in them than in Hydra? What system is present in flatworms which was entirely lacking in Hydra? Which of the systems present in complex animals like the frog are absent in planarians? What layer and what type of tissue is present in planarians that Hydra lacked? How do the muscle systems of the two animals differ? How does the process of digestion in planarians compare with that of Hydra and Protozoa? What is the essential difference between cross-sections of a planarian and of Hydra? Understand fully what parts correspond in the two animals. To what stage of embryonic development may flatworms be compared?

B. GENERAL SURVEY OF OTHER FLATWORMS

Examine the specimens provided and familiarize yourself with the appearance of the three classes of Platyhelminthes.

1. Turbellaria or free-living flatworms.—These are slender, flattened, ciliated animals living in water or damp places, often quite small, seldom of large size, similar in appearance to the type studied. To this group belong Stenostomum and other microscopic types often seen in protozoan cultures.

2. Trematoda or flukes.—These flatworms are external or internal parasites on other animals, particularly on vertebrates. They are broad, flattened, often leaflike animals, provided with suckers or hooks for adhesion to their hosts. The liver fluke,

one of the largest forms, lives in the bile ducts of sheep and other domestic animals. Several kinds of flukes are parasitic in the frog and are easily demonstrated in the living state. The chief ones are: *Pneumoneces,* the lung fluke, found inside the lungs of the frog; *Gorgoderina,* the bladder fluke, living inside the urinary bladder; and *Clinostomum,* occurring in cysts in the peritoneum. Eggs and embryos of flukes frequently occur in the frog also.

3. **Cestoda or tapeworms.**—The tapeworms are degenerate flatworms, living as internal parasites, commonly in the digestive tracts of vertebrates. They are extremely long, tapelike animals, with a small head provided with hooks or suckers or both for clinging, and a body made up of a great number of joints or *proglottids*. Each proglottid contains a complete set of the complicated reproductive organs. When ripe the proglottids drop off from the posterior end of the tapeworm and pass out in the feces.

XI. PHYLUM ECHINODERMATA

This phylum is characterized by *radial symmetry,* presence of an extensive *coelome,* an *anus,* and an *endoskeleton* in the form of calcareous plates from which spines frequently project to the exterior, and the possession of a division of the coelome called the *water-vascular system,* used chiefly for locomotion. The Echinodermata are all marine. The starfish is a familiar member of this phylum and will be studied as an example.

A. ANATOMY OF THE STARFISH

1. External anatomy.—

a) *General form and symmetry:* Examine a preserved starfish. Compare the general form of the body with that of other animals already studied. Which of these does it most resemble as to general arrangements of parts? Does the starfish have anterior and posterior ends, a head, dorsal and ventral surfaces, bilateral symmetry?

The body of the starfish is divided into two general regions, the central *disk,* and the five *arms* or *rays* which are arranged in a circle around the disk. The body also presents definite *oral* and *aboral* surfaces. The aboral surface is somewhat convex, while the oral surface is more flattened and exhibits along the center of each ray a groove containing soft objects. Both surfaces are spiny.

The starfish and all other echinoderms are characterized by the *radial* arrangements of their parts. These animals like the coelenterates are *radially symmetrical,* that is, their parts are arranged in circular fashion around a central axis, the *antero-posterior,* or *oral-aboral axis.* Whereas in Hydra, for instance, this axis is long in the starfish it is very short, and oral and aboral surfaces are much flattened, simulating ventral and dorsal surfaces somewhat as in a medusa. The radial symmetry of the echinoderms is on the plan of *fives,* while that of coelenterates is in *fours* or *sixes.* This statement means that there are five or multiples of five parts in echinoderms, four or six or multiples thereof in coelenterates spaced symmetrically around the oral-aboral axis.

Various facts demonstrate, however, that while the radial symmetry of the coelenterates is *primary,* that of echinoderms is *secondary* and acquired; in other words, they are descended from ancestors which were bilaterally symmetrical. One indication of the original bilaterality of the starfish is seen in the position of the *madreporite.* This is a small disk on the aboral surface at the edge of the disk at the angle between the bases of two of the rays. The function of the madreporite will be stated later. It will be observed that owing to the presence of the madreporite there is but one plane of symmetry, that which cuts the madreporite in half. As we learned in the study of the frog, the existence in an animal of only one plane of symmetry is an evidence of bilaterality. The two arms between whose bases the madreporite is placed are often called the *bivium,* the other three the *trivium.*

b) *External parts of the water-vascular system:* The echinoderms are characterized by a remarkable system, called the *water-vascular system,* found in no other phy-

lum, which is at present used in most echinoderms for locomotion, although its original function was probably the catching of food. The water-vascular system is composed of a system of sacs, tubes, and canals filled with a fluid consisting mostly of sea water.

Down the center of the oral surface of each ray runs a wide groove, the *ambulacral groove,* which is filled with soft projections, the *ambulacral feet* or *tube feet.* They are part of the water-vascular system. Study the arrangement of these feet. How many rows are there in each groove? Examine the shape of each foot. Each has the form of a stalk terminating in an expanded *disk* or *sucker.* The starfish walks about by means of its tube feet. The feet are stretched out for some distance beyond the body by means of water pressure in the water-vascular system, the suckers placed against the bottom, to which they adhere, the tube feet then contract, forcing the water into the internal parts of the system, and the body of the starfish is thus pulled forward. By repetitions of this process, the animal progresses slowly.

Water enters the water vascular system by means of the madreporite, a porous plate composed of calcium carbonate. Examine the madreporite with a hand lens and note that it is perforated by grooves which radiate from its center.

The remainder of the water vascular system is internal and will be seen later.

c) *The skeleton:* By this time the student will have noted that the body of the starfish is hard and spiny. This is due to the possession by the starfish of a *skeleton,* which is composed of limestone (calcium carbonate). This skeleton is of the nature of an *internal skeleton* or *endoskeleton,* like the bones of our own bodies or those of the frog.

The skeleton of the starfish consists of rods of calcium carbonate which are called *spines* when they project out through the surface and *ossicles* when they are imbedded in the body wall. Note the spines of the aboral surface. Do they show any definite arrangement? On a dried specimen scrape out the tube feet from one ambulacral groove and study the spines and ossicles of the oral surface. Observe that each side of the groove is composed of a row of ossicles called the *ambulacral* ossicles. Note that these are so shaped that pores are left between successive ossicles. These are the *ambulacral pores* through which the tube feet project. Observe alternate arrangement of successive ambulacral pores. Note the movable spines bordering the ambulacral grooves. These are the *ambulacral spines.* The groove can be contracted so that these spines interlace below it, protecting the tube feet.

From the dried specimen cut out a piece of the body wall from the aboral side of a ray, remove dried material found loose inside, and examine the skeleton from the inner view. Note that on the inside the ambulacral groove appears as the *ambulacral ridge,* formed by the ambulacral ossicles. Note that the body wall contains a network of ossicles imbedded in it. They are bound together by connective tissue and muscle fibers. Observe the thin-walled depressions between the ossicles. Determine that the spines are parts of the ossicles which project through the skin and that there are no spines over the thin places between the ossicles. Are most of the spines movable, do you think?

Draw piece of body wall from inside showing ossicles.

Return to the preserved specimen. Examine the spines of the aboral surface and note a thickened ring of skin around the base of each spine. This ring is the free edge of

the skin formed when the spine burst through the skin. Note with a hand lens little white objects in this ring of skin. Remove some of these with a fine forceps, mount in a drop of water, cover and examine under the microscope. The objects on the slide with black bodies (ossicles) in them are the structures in question. They are called *pedicellariae* and have the form of pincers or pliers. In our common Atlantic coast starfish, there are two kinds of them, those with straight jaws and those with crossed jaws. See if you can find both kinds. Note that each contains three ossicles (black spongy-looking bodies), a *basal* piece and the two *jaws*. Note muscles which operate the jaws. The ossicles are set in tissue which is part of the body wall of the animal. The pedicellariae in life open and close their jaws when stimulated and serve to catch small objects which may touch the surface of the starfish.

Draw pedicellariae.

d) *Muscular system:* As already mentioned, there are numerous muscle fibers in the body wall, where they run chiefly from one ossicle to another. These muscle fibers are of the smooth kind, similar to those of the flatworm. There are no definite *muscles,* i.e., bundles of muscle fibers, such as we saw in the frog. There are layers of muscle fibers in the walls of the tube feet. By means of its muscle fibers the starfish is capable of considerable movement of the body in general. The rays can be bent orally or aborally, to a less extent laterally. The tube feet are very active.

e) *Respiratory system:* We have already noted that between the spines are thin areas of the body wall. Examine these areas on the aboral surface with the hand lens and note that they are covered with soft finger-like projections. Remove some with a forceps to a slide, add a drop of water, cover, and examine with the microscope. Each is hollow finger-shaped process of the body wall. They are called the *dermal branchiae* or *gills*. They are used for respiration, gases passing in and out through their walls. In dead specimens they are much collapsed and contracted, giving one no idea of their appearance in life. In life they are expanded and erect, almost as tall as the spines, imparting to the surface of the starfish a velvety appearance. Do the gills and pedicellariae occur on the oral surface of the arms?

f) *Sense organs:* The starfish is not very well provided with sense organs. Both gills and pedicellariae, also the suckers of the tube feet, possess sensory cells and are sensitive to touch, chemicals, etc. At the tip of each ray is a small red or orange spot, the *eye-spot*. It seems certain from the microscopical structure of this spot and from experiments on starfish that it is an organ of light perception.

2. **The body wall and the coelome.**—Cut out a piece of the body wall from the aboral surface of one of the rays of the preserved starfish. Note the cavity or *coelome* inside the ray containing organs. Study the piece which you cut out. It consists of an external layer or "skin," the *ectoderm,* a middle region, the *mesoderm,* containing the ossicles, also connective tissue and muscles, and an internal thin glistening membrane, the *peritoneum* or *lining of the coelome*. We perceive that these relations are exactly as in the frog and in fact, they pertain to coelomate animals in general. The ectoderm of the starfish corresponds to the epidermis of the frog and to the ectoderm or epidermis of other animals studied; the mesoderm corresponds to this same layer in the frog and the flatworm and as in those animals contains muscles, connective tissue, and as in the frog. skeleton. The coelomic lining is like that of the frog. We note that the starfish

resembles the frog in the possession of a coelome, but differs from the other animals we studied in this regard.

A coelome is defined as a definite space in the mesoderm lined by peritoneum. In a coelomate animal part of the mesoderm takes part in the formation of the body wall and the rest of it participates in the formation of the internal organs.

3. Internal anatomy.—With a strong scissors cut around the margins of each ray, keeping the cut nearer the aboral than oral surface. Lift up the aboral surface of one ray and note that the internal organs are fastened to the aboral wall by means of *mesenteries* exactly as in the frog. The large soft bodies in the interior of the rays are the *digestive glands*. Carefully remove the aboral walls of the rays by cutting through the mesenteries leaving the internal organs resting in the oral half. Then cut through the angles where the rays join the disk, noting here strong columns of the skeleton, and carefully remove bit by bit the aboral wall of the disk but *leave the middle of the disk and the part bearing the madreporite in place.*

a) *Digestive system:* Turn the animal oral side up, bend the rays aborally, and note in the center of the oral surface a circular opening, the *mouth,* surrounded by a membrane, the *peristomial membrane*. Note five pairs of movable spines around the edges of the peristomial membrane. Turn the animal aboral side up again. The interior of the disk is occupied by the large *stomach* which consists of two parts. The uppermost part from the aboral view is a thin-walled pentagonal structure which clings to the aboral wall and is frequently torn in removing this wall. This is called the *pyloric division* of the stomach. From its center a very short *intestine* extends to the body wall where it opens to the exterior near the center of the disk by a small *anus*. The intestine, if not previously torn off, may be located by raising the center of the wall of the disk which was left in place above. The anus is so minute that it cannot be detected from the outside. Attached to the intestine and lying on the aboral surface of the pyloric division of the stomach is a yellowish or brownish branched structure, called the *intestinal caecum*. The function of this is doubtful. The long bodies in each arm are the *digestive glands* or *pyloric caeca* (also called *gastric caeca* and *hepatic caeca*). Observe that the pair in each arm opens into one of the five angles of the pyloric division of the stomach by a duct, the *pyloric duct*. These caeca secrete the enzyme-containing digestive fluid of the starfish.

The other and main part of the stomach is called the *cardiac division*. It is a large, five-lobed mass below the pyloric division from the aboral view and occupying the greater part of the cavity of the disk. The lobes of the cardiac division extend out into the rays where each is attached to the ambulacral ridge by two muscles, the *retractors* of the stomach. In feeding, the cardiac part of the stomach is everted through the mouth, folds over the food, and digests it by the aid of the secretion of the pyloric caeca. The digested food is then taken into the interior. Owing to this method of feeding, the intestine and anus have little function and are hence reduced in size.

Make a large outline drawing, from aboral view, of the opened starfish and in this outline fill in the digestive tract. It is not necessary to draw in the contents of all of the rays.

b) *Reproductive system:* Remove the hepatic caeca from one ray and look for the reproductive organs. The size of these is very variable depending on the sexual state

of the starfish when killed. There are two gonads present in each ray. They are elongate soft branched organs which are attached to the sides of the ray where the ray joins the disk. During the breeding season each is almost as large as one of the pyloric caeca. At other times they are reduced to very small bodies. Each opens by a duct at the point where it is attached to the ray. The sexes cannot be determined simply by inspection of the gonads but only by a microscopical examination of a bit of the gonad mashed under a cover glass. If this is done, large rounded cells, the eggs, each with a large nucleus, will be seen, if the specimen is a female. If a male, numerous small bodies appear. In life the ripe gonads differ in color.

Draw in the reproductive organs in one ray on your outline.

c) *Internal parts of the water vascular system:* Remove the stomach and look on the inner side of the madreporite for a curved canal which leads from the madreporite to the margin of the peristomial membrane. This is the *stone canal,* so called because its walls are hardened by a deposit of calcium carbonate. This canal is accompanied by a strand of tissue formerly called the *axial sinus* and believed to be a heart. It is now called the *genital stolon* and is known to give rise to the reproductive organs. The stone canal leads into the *ring* or *circumoral canal* which runs on the inner surface of the attached margin of the peristomial membrane; but it is exceedingly difficult to see. From the ring canal, a *radial* canal is given off into each ray and runs the length of the ray imbedded in the ambulacral ridge.

In a ray from which the pyloric caeca have been removed note on the inner surface of the floor of the ray, alongside the ambulacral ridge, rows of small sacs, the *ampullae.* Observe exact number and arrangement of the rows. Each ampulla connects through the ambulacral pore between the ambulacral plates of the ambulacral ridge with a tube foot. Each ampulla also connects by a small duct or *transverse canal* with the radial canal of that ray. We thus have a continuous system from the madreporite, through the stone canal and ring canal, out along the radial canals, through the transverse canals into the ampullae, and finally into the tube feet. This system is filled with water in life which is brought into the system by a ciliary current in the madreporite and stone canal. As already stated, this system is used in locomotion. The tube feet are extended by contractions of the ampullae which force the contained fluid down into the tube feet. The suckers of the tube feet then take hold, and the feet contract pulling the starfish forward while the fluid in the feet flows back into the ampullae. The system is not quite water-tight; hence the necessity for an inlet through the madreporite to maintain the necessary quantity of water in the system. There is a pair of valves in each transverse canal which prevents the water from flowing back into the radial canal from the ampullae.

It is difficult to see the parts of the above system except the ampullae and tube feet. The radial canal is best seen by cutting across a ray. It appears in the section as a small hole near the bottom of the ambulacral groove just under the skin. By other cuts across the ray the connection of ampulla and foot may sometimes be found. All of these details are best observed in specimens in which the water-vascular system has been injected with a colored fluid.

Make a diagram of the arrangement of the water-vascular system.

On the wall which bounds the cavity of the disk may be noted nine small bodies, the stone canal occupying the place where a tenth would be expected. These bodies are called the *racemose bodies* or *Tiedemann's bodies*. They are said to produce amoeboid cells which wander about in the coelomic spaces. They connect with the ring canal of the water-vascular system.

d) *Nervous system:* There is fine network of nerve tissue consisting of nerve-cell bodies and their processes just underneath the ectoderm over the entire body of the starfish. This arrangement is very like the form of the nervous system in coelenterates. In certain regions of the starfish, this nervous network is sufficiently thickened to be visible to the naked eye as a "nerve." This thickened part of the nervous system consists of a *ring* or *circumoral nerve* and five *radial nerves*, all located on the oral surface. Each radial nerve runs along the bottom of the ambulacral groove. If the two sides of the ray be bent aborally so as to expose the bottom of the ambulacral groove, a cord, the radial nerve, will be seen running the length of the groove. It terminates at the end of the ray in the eye-spot already noted. A similar thickening in the form of a nerve ring will be found on the oral surface of the peristomial membrane at its margin just oral to the ring canal of the water vascular system.

e) *Other systems:* The starfish does not possess any circulatory system. The coelomic cavities, which include not only the general coelome, but also the water-vascular system, and several other systems of spaces, are filled with a fluid which probably moves about more or less. This fluid contains wandering amoeboid cells, which resemble the white blood corpuscles of vertebrates.

There is likewise no excretory system. The amoeboid cells just mentioned are said to pick up waste matters; they then pass to the outside by way of the dermal branchiae and disintegrate. Presumably also waste substances may pass out directly through body surfaces.

4. General considerations on the starfish.—In the starfish we meet for the first time since we began to ascend the scale of animal life with a coelome. The student should be able to see that the relations of the coelome are exactly the same as in the frog. It is a space lined by a peritoneal membrane; it separates the body wall from the digestive tract. The body wall has the same composition that we saw in the frog and if we were to study histologically the structure of the wall of the stomach we should find it composed of layers similar to those of the frog's intestine. The starfish is, therefore, a *triploblastic* animal like the flatworm and, in addition, a *coelomate* animal like the frog. The appearance of a coelome is the next stage in the development of complexity of structure after the appearance of the mesoderm.

Does the starfish possess a definite head as compared with Planaria? With what animal that we have studied is it most comparable as to head? Is the lack of a head related to radial symmetry? Do you think it possible for a radially symmetrical animal to develop a definite anterior end? Does this explain why the great majority of animals are bilaterally symmetrical?

What systems does the starfish possess that were lacking in coelenterates and flatworms? What systems does it still lack compared with an animal like the frog? With what animal previously studied is the nervous system most comparable? Would you consider a nervous system of this type efficient in meeting the conditions of life?

B. GENERAL SURVEY OF ECHINODERMS

There are five classes of this phylum, four of which are very common animals of the sea.

1. Asteroidea.—This is the class to which the starfish belongs. There are a great many different kinds of starfish, varying chiefly in relative proportions of disk and arms, and arrangement of spines and ossicles. They generally possess five rays but some are regularly six-rayed and some have a large number of rays, as the so-called twenty-rayed star of the Pacific coast. Some of these kinds of starfish will be shown to you if available.

2. Ophiuroidea.—These forms are known as brittle-stars or serpent-stars. They are mostly of small size, with a rounded central disk and five slender arms. These arms are capable of much movement and in fact the animal progresses by writhing movements of its arms, the tube feet being degenerate. The basket-star, a large form with much branched arms, one of the most striking of our marine animals, belongs in this group of echinoderms. It lives in fairly deep water.

3. Echinoidea.—The echinoids include a number of bizarre-looking animals of the seashores, known as sea-urchins, heart-urchins, and sand-dollars. They vary in shape from nearly spherical to flat forms, and all are markedly spiny. The sea-urchins, the most common of these animals, have hard rounded bodies with many long spines projecting from the body. The tube feet are seen to be disposed in five rows which run from the oral surface to the aboral pole. In the center of the oral surface is seen the mouth, occupied by five protruding teeth which are part of an apparatus for chewing seaweeds. Around the mouth is an extensive peristomial membrane. The animal moves about on its tube feet as does the starfish. If the spines and soft skin are rubbed off from the sea-urchin its internal skeleton, called the *test*, is exposed. It consists of flat broad ossicles joined together in a definite pattern. The sand-dollars are flattened animals covered with short spines. On the oral surface are five grooves in which occur the walking tube feet; while on the aboral surface are located special modified tube feet, used for respiration and arranged in five areas like the petals of a flower.

4. Holothuroidea.—The holothurians are commonly known as sea-cucumbers on account of the shape of their bodies. They are somewhat unlike other echinoderms in superficial appearance, having tough leathery elongated bodies, with the mouth surrounded by branched tentacles. Spines are lacking. In some forms the tube feet are arranged in five rows as in starfish and sea-urchins while in others they are scattered over the body.

5. Crinoidea.—The crinoids or sea-lilies are among our most beautiful marine animals but are not very common and live in fairly deep water. Most of them are sessile, being attached to the bottom by a slender stalk. At the upper end of this stalk is fastened the body or *calyx* of the animal from which spring branched arms. The oral surface is held uppermost in these animals, the aboral surface attached to the stalk. Some crinoids have lost the stalk and are able to move about by muscular movements of the arms, but still hold the oral surface upwards. The present-day crinoids constitute the small remnant of a very large group of extinct animals, from which the other existing types of echinoderms were derived.

XII. PHYLUM ANNELIDA

The phylum Annelida (or Annulata) is composed of the segmented worms, a group of animals whose most characteristic structural feature is the *segmentation* of their bodies, both *external* and *internal*. Other characters are: *triploblastic* structure, presence of an *anus*, and usually of a well-developed *coelome*, and possession of a *circulatory system*. The annelids inhabit fresh and salt waters and most kinds of soil.

A. PRELIMINARY STUDY OF NEREIS

Nereis is an annelid living in the mud in the shallow waters of the ocean. It is a typical annelid in many respects and is hence used to illustrate certain points about this phylum. The dissection, however, will be performed upon an earthworm, a somewhat more specialized annelid, but one more readily dissected.

1. External anatomy of Nereis.—Place a preserved specimen in a dissecting pan. Compare general form and symmetry with animals previously studied. Is Nereis bilaterally symmetrical? The most striking feature of the body is its division into a longitudinal series of rings, called *segments*, *metameres*, or *somites*. An animal so constructed is said to be *segmented*. Segmentation is the next advance in complexity of structure in the animal series we are studying. Each segment bears a pair of broad lateral outgrowths, the *parapodia*, used in swimming. Distinguish anterior and posterior ends, and dorsal (rounded) and ventral (flattened) surfaces.

a) Head: The first segment of the body is markedly differentiated as a *head*, which bears a number of *sense organs*. (In most specimens the large *pharynx* bearing jaws will be found protruded from the mouth so as to extend some distance anterior to the head. This should not be confused with the true head.) The head segment consists of two parts, a dorsal squarish projection, the *prostomium*, which does not extend to the ventral side, and just behind this a complete ringlike segment, the *peristomium*, which surrounds the mouth.

The sense organs of the head consist of *tentacles*, *palps*, and *eyes*. There are two short *terminal* tentacles projecting from the middle of the anterior edge of the prostomium. On each side of these is a thick jointed palp. On the dorsal surface of the prostomium are four eyes, indistinct blackish spots so situated as to form the four corners of a trapezoidal area. The two posterior eyes are likely to be concealed under the anterior edge of the peristomium. The eyes of Nereis are much more complex than those of Planaria and are provided with lenses so that the animal can probably distinguish objects in a dim way. The peristomium bears on each side four conspicuous, long, *lateral* tentacles or *cirri*. It is probable that tentacles and palps are organs of touch, contact, chemical sense, etc. It is known that they are thickly strewn with sensory cells. **Make a drawing of the head enlarged.**

The head of Nereis represents another step in advance in the series of animal forms which we are considering in that it is more sharply differentiated from the rest of the

body than is the case in coelenterates, flatworms, or echinoderms and is provided with a greater variety of sense organs and more complex and sensitive ones.

b) *Posterior end:* The last segment differs from the body segments in the absence of parapodia and the presence of two long tentacles or *cirri*, of sensory function. The last segment also bears a central opening, the *anus*. The presence of an anus marks another advance over coelenterates and flatworms in which animals the digestive tract has but one opening to the exterior, the mouth.

c) *Remainder of the body:* Note that with exception of the first and last segments all the segments of the body are alike, omitting minor differences in the size and shape of the parapodia. This similarity of segments is not merely superficial but extends to all of the details of internal anatomy.

d) *Parapodium:* Parapodia are characteristic of an entire group of annelids, the group known as Polychaeta. Cut one off with a scissors, mount in water, cover, and examine with low power. It consists of several lobes, one of which, the *gill plate*, is large and leaflike and serves as a respiratory organ. Observe the stiff bristles, *setae*, or *chaetae*, which project from the parapodium. **Draw.**

B. ANATOMY OF AN EARTHWORM

The earthworms are annelids somewhat more specialized than Nereis. There are a number of different kinds of earthworms of which the large form, *Lumbricus terrestris*, is usually chosen for dissection. The following directions apply specifically to this species; other species differ as to location of sex organs, sexual pores, clitellum, etc.

1. **External anatomy.**—Obtain a preserved specimen, and compare with Nereis as to differentiation of a head, form, symmetry, segmentation, etc. Are the two animals similar? Distinguish anterior (more pointed) and posterior ends, dorsal (dark-colored, rounded), and ventral (light-colored, flattened) surfaces. Is the head as well developed as in Nereis? Is this related to the habit of life of earthworms? The head of Nereis is to be regarded as a typical annelid head while that of the earthworm is degenerate. Are parapodia present?

a) *Head:* The first segment is composed as in Nereis of a *prostomium*, the liplike process dorsal to the mouth opening, and the *peristomium*, the ring surrounding the mouth. The head of the earthworm probably consists of the first four or five segments as indicated by experiments in regeneration but is not marked off morphologically. Does the head bear eyes or other visible sense organs? Numerous sensory cells are imbedded in the surface layer.

b) *Clitellum:* Anterior to the middle of the worm a number of swollen segments occur, producing a distinct girdle, the *clitellum*, around the body. The clitellum is a glandular region which secretes the cocoon in which the eggs of the earthworm are laid. How many segments are there in the clitellum? How many segments anterior to the first clitellar segment? Is this number the same for all individuals? Remember that the prostomium is not a segment.

c) *External openings:* The mouth opening was already noted. The last segment contains the *anus*. On the ventral surface of the fifteenth segment is a pair of conspicuous openings with swollen lips, the *male pores*. The smaller *female pores* are on the

fourteenth segment and can usually be seen in large specimens. Other openings present but too minute to see are: the openings of the two pairs of seminal receptacles ventrally in the grooves between the ninth and tenth and tenth and eleventh segments; a pair of excretory openings on the ventral surface of each segment, except the first three and the last; and dorsal openings, the dorsal pores.

d) Setae: The earthworm, like Nereis, bears setae. These are arranged in four double longitudinal rows, two ventral and two lateral. Pass your finger up and down the worm and feel the setae as rough projections. With a hand lens make out their arrangement, the anterior part of the body being a favorable place to examine. They are used by the worm to prevent slipping. **Make a diagram,** representing a segment as a ring and show the position of the setae on the ring.

2. General internal structure.—The earthworm is a typical *triploblastic coelomate* animal. This means that it consists of two tubes, an outer one, the *body wall,* composed of ectoderm and mesoderm, and an inner one, the *digestive tube,* composed of entoderm and mesoderm, with a *coelomic cavity,* that is, a cavity in the mesoderm lined by a definite mesodermal membrane, the *peritoneum,* between these two tubes. A further structural feature of the earthworm is its *segmentation.*

a) Characteristics of the coelome: With a scissors make a cut about an inch long through the dorsal wall behind the clitellum. Be careful to cut only the body wall; if black material oozes out you may know that you are cutting into the intestine and should withdraw your scissors and begin again less deeply. Separate the edges of the cut and look inside. Observe that the *body wall* is separated from the central dark tube, the *intestine,* by a distinct space, the *coelome.* Observe further that this space is not continuous but is divided up into a series of compartments by delicate white partitions, the *septa* or *mesenteries,* which extend at intervals from the body wall to the intestine. What is the relation of these septa to the external rings? The coelome of the earthworm is thus *segmented,* that is, divided up on each side into a longitudinal series of chambers. The anterior and posterior walls of each chamber come in contact with the walls of those in front of and behind it producing double-walled partitions, the septa already noted. The outer wall of each chamber is fused to the inside of the body wall forming a membrane called the *parietal peritoneum.* This is the smooth shining membrane lining the body wall. The inner wall of each coelomic chamber is similarly fused to the outer surface of the intestine forming a layer (not distinct to the naked eye) called the *visceral peritoneum.* The inner walls of the chambers of the two sides inclose the intestine between them forming double-walled membranes in the mid-dorsal and mid-ventral lines. These are called the *dorsal* and *ventral mesenteries* of the intestine, and with the septa hold the intestine in place. The dorsal mesentery is present only at the septa while the ventral is continuous.

b) Characteristics of the body wall: The body wall is covered externally by a thin iridescent *cuticle,* which is a lifeless, non-cellular secretion. Strip off a small piece of its mount in water, and examine with high power. It shows striations which cause the iridescence, and numerous pores, the openings of gland cells. Draw a small portion of cuticle. Examine the cut surface of the body wall of your specimen with a hand lens. Note outer thin whitish layer, the *epidermis,* which secretes the cuticle, and internal to

this a thicker greenish layer composed of *muscles*. Next comes the parietal peritoneum. The body wall is thus composed of three layers—epidermis, muscle layer, and peritoneum—as in the frog, starfish, and coelomate animals in general.

c) *Principal organs:* Extend your cut forward slightly to the right of the mid-dorsal line up to the prostomium. Beginning at the posterior end of your cut, stick a pin in the right body wall. Cut through the nearby septa of the right side. Then lift up the other edge of the cut, cut through the septa of the left side, and pin this side out. Move forward a short distance and repeat. Continue anteriorly to the prostomium. Stick the pins in obliquely, heads away from the animal. Do not pull the walls apart but cut through the septa with a fine scissors. *Cover the animal completely with water.*

The principal organs may now be identified. The center of the body is occupied by the long brownish *digestive tract*. In the tenth to twelfth segments are several large white bodies, the *seminal vesicles*, parts of the male system. Just anterior to them are two pairs of small white spherical bodies, the *seminal receptacles*, belonging to the female system. The loose white objects alongside the digestive tract are the *excretory organs* or *nephridia*.

3. Systems of the earthworm.—The earthworm has most of the systems characteristic of complex animals (see p. 9).

a) *Muscular system:* This consists of muscles in the body wall, arranged chiefly in the form of *longitudinal bands*. The most conspicuous of these are the paired *dorsal bands*, extending from the mid-dorsal line to about the middle of the lateral wall on each side. Other bands will be noted later.

b) *Circulatory system:* A well-developed circulatory system of the nature of a *blood-vascular system* is present in the earthworm, consisting of vessels connected by capillaries. This system is, like that of the frog, of the *closed* type, that is the vessels form a continuous circuit and do not open into body spaces. Owing to the small size of the vessels, this system is difficult to study and only the principal parts are mentioned here.

(1) *Dorsal vessel:* This is the brownish tube running along the mid-dorsal line of the intestine. It receives in each segment two pairs of *dorso-intestinal* vessels from the intestine and a pair of *parietals* (torn across, of course) from the body wall. These can be seen in some specimens.

(2) *Hearts:* Remove the seminal vesicles from the right side. Note that in the region where they were located and for some distance anterior to this the septa are stronger and more conspicuous than elsewhere, projecting out from the intestine like wings. Look in the eleventh segment between the places which were occupied by the second and third pairs of seminal vesicles for a pair of stout dark-colored tubes extending from the dorsal vessel ventrally. Then look in each segment forward from this up to the sixth for a similar pair of tubes situated just in front of the winglike septa which should be pulled backward to see them. These five pairs of vessels extending ventrally from the dorsal vessel are contractile tubes, called *hearts*. Follow the dorsal vessel forward as far as you can anterior to the region of the hearts.

(3) *Ventral vessel:* Loosen up the intestine completely on the right side and press

it over to the left so that you can see the mid-ventral line. Look here on the ventral surface of the intestine for a brownish line, the *ventral vessel.* It is easiest to see under the dilated part of the digestive tract behind the region of the seminal vesicles. Look for branches from the ventral vessel into the intestine, body wall, and nephridia. Trace the connection of the hearts with the ventral vessel and follow the latter forward as far as you can.

(4) *Subneural vessel:* In the mid-ventral line of the body wall is a white cord, the ventral nerve cord. Cut through this at some point back of the region of the hearts, pull up a short strip of it backward, and look on its under surface for a vessel, the *subneural* vessel, looking like a dark line along the white cord. Its branches to the body wall may sometimes be found.

(5) *Intestino-tegumentary vessels:* In the tenth segment an extra vessel arises on each side from the dorsal vessel and extends forward along the wall of the digestive tract. Hearts and other intervening structures may be removed on the right side to see it.

Make a drawing from the side of the parts of the circulatory system which you were able to find. Some specimens will show more of the vessels than others.

On living earthworms observe the rhythmic contractions of the dorsal vessel. In which direction do the contractions travel? Find out from texts the course of the circulation in the earthworm.

c) Digestive system: This is a continuous tube from mouth to anus differentiated into several parts. A small tube called the *buccal pouch* extends from the mouth through about three segments. It is attached to the body wall by muscle fibers, the *dilators,* which help it expand in food ingestion. On its dorsal surface at its junction with the next region of the digestive tube note a small white bilobed object, the *brain.* Following the buccal pouch is the *pharynx,* thick-walled, with numerous dilator muscle fibers. From the pharynx a slender tube, the *esophagus,* extends through the region where the hearts and seminal vesicles are located. These should be removed on the right side, if not already done, to reveal the esophagus. In its wall in the region of the seminal vesicles are three pairs of brownish projections, caused by the *calciferous glands.* These glands are reputed to secrete calcium carbonate into the digestive tract. The esophagus leads into a large thin-walled *crop,* where food is stored. Immediately posterior to the crop is the equally large thick-walled *gizzard* in which the food is ground to small particles. From this the brown thin-walled *intestine* extends to the anus. Cut open the intestine along one side for a short distance, wash it out, and note a thickening caused by a fold in its mid-dorsal line. This fold is called the *typhlosole* and probably serves to increase the absorptive surface of the intestine.

Draw from dorsal view showing outline of the worm and parts of the digestive tract in relation to the segments, numbered.

d) Excretory system: The excretory system consists of a pair of coiled tubes, called *nephridia,* present in each segment except the first three and last. The nephridia are the loose white structures seen to the sides of the digestive tract. Extend your original cut about one inch backward, this time exactly in the mid-dorsal line. Pin out the body wall as before. Remove the intestine at this place. Observe with a hand lens the

paired nephridia, each consisting mainly of a tube transversely coiled in the coelome of each segment. Each nephridium begins, however, in the segment preceding that in which its main part lies, forming there a *funnel* or entrance into the nephridium. This appears as a small white body. From the funnel a tube penetrates the septum behind and passes into the coiled portion. Cut out a nephridium as completely as possible, mount on a slide in water, examine with low power, and compare with figures in texts, identifying such parts as you can.

This excretory system of the annelids illustrates the fundamental plan on which the excretory systems of most of the higher animals are built. It is seen to consist of tubes leading from the coelome to the exterior.

e) *Reproductive system:* The dissection of this system is difficult and should be attempted only on large well-preserved specimens and with the aid of good figures.

As in flatworms, a complete set of both male and female organs is present in the earthworm, that is, it is *hermaphroditic*. The left side of the animal has been preserved intact for this dissection. Remove the esophagus from the region of the seminal vesicles (no farther) and study the latter on the left side. The first pair are rounded sacs projecting forward into the ninth segment. The second vesicles are more elongated and folded and occupy the eleventh segment. The third vesicles are the largest and are much folded, occupying the twelfth and part of the thirteenth segments. The three pairs are united by median masses located in the tenth and eleventh segments. Locate the fifteenth segment and stick a pin into it as a landmark. Gently press the vesicles to the right and pull off the nephridia from the left ventral body wall by grasping their loose portions. Stretch the left ventral wall out tightly and pin it down. Locate the ventral edge of the dorsal longitudinal muscle band and just medial to this the lateral setae projecting inward as minute white elevations. One-third of the way from these setae to the mid-ventral line find a white line extending from the fifteenth segment anteriorly to the tenth. This is the left *vas deferens* or *male duct*. Examine the septum between the thirteenth and fourteenth segments. The white object in it is the left *oviduct* which continues as a slender tube into the fourteenth segment. Then examine the septum between the twelfth and thirteenth segments. A minute white conical body, the left *ovary*, is attached to it, its pointed free end projecting posteriorly into the thirteenth segment. To prove that it is an ovary remove and examine with the microscope; it should be filled with round eggs, the largest in the pointed end. Next look in the twelfth segment for a slender branch extending from the vas deferens over to the median part of the seminal vesicles. Dissect off the roof of the median mass of the seminal vesicles in the eleventh segment and note in its cavity the greatly folded *seminal funnel*. Anterior to it, attached to the septum between the tenth and eleventh segments is situated a small round body, the posterior *testis*. This is very difficult to find, but an attempt to locate it should be made by picking away the seminal vesicle in the region indicated. Repeat the directions in the preceding four sentences and try to find the anterior branch of the vas deferens, the anterior seminal funnel, and the anterior testis, attached to the septum between the ninth and tenth segments. Examine contents of the seminal vesicles and seminal funnels under the microscope. Balls of cells which are stages in the development of spermatozoa, and myriads of the mature long slender *spermatozoa* will

be seen. The mother-cells of the sperm (spermatogonia) are produced by the testes but are set free at an early stage into the seminal vesicles, in which they develop into spermatozoa. The two rounded *seminal receptacles* were previously identified. They receive the sperm from another animal during copulation and are, therefore, parts of the female system. Read in texts on copulation, cocoon formation, and egg-laying in earthworms.

Draw the reproductive system.

f) Nervous system: Remove the digestive system as far forward as the posterior end of the pharynx. The *ventral nerve cord*, a white cord in the mid-ventral line, was previously identified. Clean away all tissue concealing it. Examine it with a hand lens and note the enlargement or *ganglion* formed by the nerve cord in the center of each segment, and the *lateral branches* springing from cord and ganglia. Trace the nerve cord anteriorly. Loosen the pharynx and turn it with its posterior end forward. Find underneath it the anterior termination of the ventral nerve cord in a large double mass, the *sub-esophageal ganglia*. Locate the brain on the dorsal side of the buccal pouch and cut away the pharynx and as much of the buccal pouch as you safely can, leaving the brain in place. Note the two cords which extend dorsally from the sub-esophageal ganglia to the brain, forming a circle through which the buccal pouch passes. These cords are called the *circumesophageal connectives*. Examine the brain and observe that it consists of two lobes, the *supra-esophageal ganglia*. Look for nerves running from the brain to the prostomium and from the connectives to the ventral parts of the first segments.

Draw the nervous system.

Strip off a piece of the ventral nerve cord from the anterior part of the worm, mount in water on a slide, and examine with low power. Determine on this piece how many lateral nerves pass out from each ganglion and how many arise from the nerve cord between ganglia. Add these branches to your drawing. The subneural blood vessel and its branches will probably be noted on the ventral surface of the cord; they are yellowish and hollow and should not be confused with nerves which are white and solid, with longitudinal striations.

4. **Histological structure of the earthworm.**—Examine a prepared cross-section through the earthworm.

a) General features of the cross-section: Examine with low power. Identify the thick body wall, the central intestine with its dorsal infolded typhlosole, and the coelome between the body wall and intestine. In the body wall the most conspicuous feature is the feathery layer of longitudinal muscles interrupted in eight places for the insertion of the setae. These interruptions divide the longitudinal muscle coat into bands, as follows: the *dorsal* bands extending from the mid-dorsal line to the lateral setae; the *lateral* bands between the lateral and ventral setae; and the single *ventral* band across the ventral side. There are also small bands between the two members of each pair of setae. Identify the dorsal blood vessel lying above the typhlosole; the ventral vessel below the intestine to which it is attached by the ventral mesentery; and the ventral nerve cord near the ventral vessel. In the coelome may be seen traces of nephridia, blood vessels, and septa. **Make a diagram of the section.**

b) *Structure of the body wall:* With high power study the layers of the body wall, proceeding from the external surface inward.

(1) *Cuticle,* the outermost layer, a thin non-cellular membrane.

(2) *Epidermis:* This is a layer of epithelial cells of ectodermal origin. It consists mainly of the *gland* cells, large elliptical epithelial cells filled with granules which are forerunners of mucus. These gland cells open on the surface by way of the pores through the cuticle already noted. Between the gland cells are non-glandular epithelial cells, called the *interstitial cells.* At the bases of the gland and interstitial cells may be noted some small cells, which are sometimes considered to constitute a second layer of the epidermis. The epidermis contains numerous sensory cells, not visible without special methods of staining.

(3) *Circular muscle layer:* This consists of very long slender smooth muscle cells running circularly, imbedded in connective tissue.

(4) *Longitudinal muscle layer:* This is composed of muscle cells like those of the circular layer but as they run longitudinally, they appear in cross-sections as circles or ellipses. These longitudinal muscle cells are mounted on plates or septa of connective tissue which extend at right angles to the body wall. Each such plate bearing on both surfaces cross-sections of muscle cells looks like a feather. The interruptions of the longitudinal muscle layer for the setae were already noted.

(5) *Peritoneum:* This is the thin layer applied to the inner surface of the longitudinal muscle layer.

(6) *Setae:* If setae are present on your slide note their appearance. They are slender, yellow, curved rods of the same composition as the cuticle and like it secreted by the epidermis. The epidermis turns in to form a sheath around each seta and a cap at its inner end. This cap secretes the seta. From the epidermal sheath and cap muscles extend to the circular layer and serve to move the seta in all directions.

Draw a portion of the body wall in great detail.

c) *Structure of the intestinal wall:*

(1) *Chlorogogue layer:* The outer layer of the intestine consists of very large, irregular, pear-shaped cells, the chlorogogue cells. This layer represents the *visceral peritoneum,* highly modified. The function of the chlorogogue layer is uncertain.

(2) *Longitudinal muscle layer,* a thin layer appearing as circles at the attached ends of the chlorogogue cells.

(3) *Circular muscle layer,* a thin layer of circularly arranged muscle cells internal to the preceding.

(4) *Vascular plexus:* The blood vessels of the intestinal wall form a rich network or *plexus* situated just internal to the circular muscle layer, appearing in cross-section as irregular spaces, uniformly stained.

(5) *Lining epithelium:* The innermost layer of the intestinal wall, lining the cavity, is an epithelium, composed of a single layer of columnar ciliated epithelial cells, about as wide as the chlorogogue layer. The nuclei of these cells are near their attached ends.

Draw a portion of the intestinal wall in great detail.

d) *Structure of the nerve cord:* The nerve cord is an oval object ventral to or to

one side of the ventral vessel. Examine with high power. It is covered externally with a sheath consisting of peritoneum, connective tissue, muscle cells, and blood vessels. Of these latter the more conspicuous ones are the *subneural* vessel below the middle of the cord, and the paired *lateral neural* vessels, one on each side of the cord.

The nerve cord is more or less distinctly divided into two halves by a median partition. The cord is thus double, consisting in reality of two originally separate cords which during development fused together. The dorsal part of the cord consists of three large clear areas, the *giant fibers*, consisting of nerve fibers. Each is surrounded by a thick sheath. In the lateral and ventral portions of the cord may usually be seen several large pear-shaped *nerve cells*, each with a large nucleus and nucleolus. The nerve cells are more abundant if the section has happened to pass through one of the ganglionic swellings of the cord. The rest of the cord consists of cross-sections of nerve fibers, appearing like a network.

Draw the section of the nerve cord.

e) *Longitudinal section of the earthworm:* Study and identify the parts already seen in cross-section. Note particularly the septa.

5. Behavior of the earthworm.—Freshly dug earthworms should be used if possible. They must be handled gently.

a) *Locomotion:* Put a thin layer of moist earth over the bottom of a large pan. Place an earthworm in the pan, keep away from strong light, and observe carefully just how the earthworm crawls and how the setae are used. Explain rôle of the circular and longitudinal muscle coats of the body wall in crawling. Understand that contraction of circular muscles diminishes the circumference and increases the length of the body while contraction of longitudinal muscles has the opposite effect. Observe behavior of the anterior end and prostomium in crawling. Does the animal leave a trail of slime?

b) *Burrowing:* Place some earthworms on a layer several inches thick of moist earth of the same kind as that from which they were dug, keep away from strong light, and try to observe process of burrowing.

c) *Response to mechanical stimulus:* Place an earthworm in a pan having a thin layer of moist earth or a sheet of damp filter paper on the bottom, set the pan away from strong light, and after the worm has recovered from handling, try its response to touch. Use a glass rod drawn to a fine but blunt point or some similar instrument. Wait for the animal to recover between successive touches. What does the animal do when touched? Try the effect of a touch of equal strength applied successively to anterior, middle, and posterior thirds. Is the response different according to the part of the body touched? Try a light and strong touch applied to the same region and note any differences in response.

d) *Response to moisture (hydrotaxis):* Put a thin layer of dry earth or a sheet of dry filter paper in the bottom of a pan. Wet a large spot in the center leaving the rest dry. Place an earthworm on the wet spot and note whether it shows any response when its head comes in contact with the dry region. Do you think earthworms are positively or negatively hydrotactic?

e) *Response to contact (thigmotaxis):* Place several earthworms in a pan having damp earth or moist filter paper on the bottom and note after a time if the worms show

any tendency to aggregate in a bunch. Place a single worm in such a pan with objects such as stones, leaves, etc., and note after a time if the worm shows any response to such objects. Arrange on the bottom of such a pan two suitable objects so that a space of the general shape of a burrow is left between them. Direct the anterior end of a worm toward the entrance of the artificial "burrow" and observe what the worm does. Is the earthworm negatively or positively thigmotactic?

f) Reaction to light (phototaxis): Place a worm in a pan with moist bottom and keep in darkness for half an hour. Expose suddenly to bright light, preferably sunlight, and note any reaction. Distribute several earthworms equally over the moist bottom of a pan, cover pan with a glass plate, then cover half of the plate so that one half of the interior of the pan is darkened, and set in the light. After some time note whether the worms show any tendency to collect in either the dark or light half. Is the earthworm positive or negative to light?

6. General considerations.—The principal advances in structure illustrated by annelids are the presence of a coelome and of segmentation. These two important features may now be defined. A coelome is a definite space in the mesoderm lined by a membrane, the peritoneum, which bears the reproductive organs and which forms the inner lining of the body wall, the outer coat of the digestive tract and other viscera, and the mesenteries which support the viscera. Segmentation is the repetition of structures at regular intervals along the antero-posterior axis. In the study of the anatomy of the earthworm, we have seen that nephridia, nerve ganglia, branches of the blood vessels, setae, septa, are repeated at definite intervals, each such interval constituting a segment.

The earthworm and other annelids also possess one system not found in any of the animals hitherto studied (except the frog). What is it? Which of the systems found in a complex animal like the frog are still lacking in the earthworm? How does the digestive system of the earthworm differ from that of Planaria? How do the excretory systems of these two animals differ? What system of the earthworm shows the most resemblance to that of Planaria? What striking differences exist between cross-sections of Hydra, Planaria, and the earthworm? In making such a comparison understand that the cavity of the digestive tract of the earthworm is the same as the gastrovascular cavity of coelenterates and flatworms; the lining epithelium of the digestive tract is the entoderm; the epidermis is the ectoderm; and all of the tissue between is mesoderm. Is the histological structure of the body wall and the intestinal wall of the earthworm similar to that of the frog?

C. GENERAL SURVEY OF ANNELIDS

1. Polychaetes.—The polychaetes are the group of marine annelids to which Nereis belongs. They are characterized by the presence of numerous setae usually borne on parapodia. Examine various specimens. Note form and segmentation of the body, development of parapodia, presence and location of gills (filamentous processes often arranged in clumps), degree of development of the head. Many polychaetes live in

tubes secreted by themselves, and hence the parapodia and head regions are often degenerate.

2. **Oligochaetes.**—The earthworms and certain small segmented worms living in fresh waters belong to this group. They are all very much alike, characterized by the lack of parapodia, small number of setae, and lack of external sense organs on the head.

3. **Hirudinea or leeches.**—These common annelids of fresh waters are easily recognized by the presence of two suckers, one at each end of the body. They are external parasites sucking the blood of vertebrates. Are parapodia or setae present?

XIII. PHYLUM MOLLUSCA

The phylum Mollusca includes such animals as oysters, clams, snails, mussels, slugs, cuttlefish, squids, and octopuses. The most important features of the phylum are: an *exoskeleton* in the form of a calcareous *shell;* a fold of the body wall called the *mantle* which secretes the shell; and the modification of the ventral part of the body into a locomotor organ, the *foot.* There is no segmentation; a coelome is present but usually greatly reduced; there are well-developed circulatory, excretory, and respiratory systems.

The snails are typical representatives of this phylum but as they are somewhat difficult to dissect will be used to illustrate external anatomy chiefly. A clam will serve better for the study of the internal anatomy.

A. ANATOMY OF A SNAIL

For this purpose either living or preserved extended specimens of our common land or pond snails may be used.

1. General features.—On examination of a living or preserved snail one notes at once that the animal bears an external skeleton or *exoskeleton* in the form of a *shell.* Part of the animal is concealed within this shell and part of the animal protrudes from it. Observe that the shell is *spirally coiled.* A spiral arrangement is derived from a bilateral arrangement. Is the part of the animal which protrudes from the shell bilaterally symmetrical? Does it have a definite head? posterior end? The flattened ventral surface on which the animal crawls is named the *foot.* The shell is situated dorsally on the animal and incloses most of the dorsal part of the body.

2. Shell.—A variety of empty snail shells should be on hand. Note various shapes, colors, protuberances, etc. The shell of snails is nearly always spirally coiled and is generally of the shape of a long or short cone, but is in some cases flattened to a disk. The parts of the shell are named as follows: the beginning of the spiral is called the *apex,* the turns are known as *whorls,* the last and largest whorl is termed the *body whorl,* and the opening of the shell is designated the *mouth* or *aperture.* The margin of the aperture is known as the *lip* and in many marine snails is drawn out into a spoutlike projection, the *siphonal canal,* used in respiration. In a broken shell, note the central axis, or *columella,* around which the whorls are wound. The surface of the shell is marked by lines, the *lines of growth,* which indicate rate and direction of growth. Note places particularly on the shells of fresh-water snails where the lines of growth are very close together making a deep mark. Can you suggest any external conditions which would cause such a slow rate of growth at intervals? The apex is the oldest part of the shell, the body whorl, the youngest; the shell increases in size by additions at the lip.

The shell is composed of an outer thin layer, called the *periostracum,* made of a highly insoluble organic material; and of a thick inner *calcareous* layer, made of lime-

stone (calcium carbonate). Put a shell in hydrochloric acid and observe what happens. Which part dissolves and which is left?

Draw a shell, labeling its parts.

3. **Head.**—The head is relatively large and well developed. It bears one or two pairs of *tentacles,* which are organs of touch and probably chemical sense. There is a pair of *eyes,* situated either at the base, or at the sides, or at or near the tips of the tentacles. They appear to the naked eye as black dots. These eyes are much more complex in structure than those of a flatworm or starfish but are somewhat like those of Nereis. The sides of the head may be expanded into lobes, the *labial palps,* of sensory functions.

The *mouth* is situated on the ventral surface of the head. It is a simple opening in our fresh-water and land snails but in many marine forms, a cylindrical tube, the *proboscis,* projects through the mouth. This proboscis is a part of the digestive tract everted to the outside.

4. **Foot.**—The ventral part of the snail is expanded into a muscular structure, broad in front, tapering to a point behind, and flat ventrally. This structure is known as the *foot* and is a characteristic feature of mollusks. On the dorsal surface of the posterior end of the foot is situated in some snails an oval or circular piece of shell called the *operculum.* This serves to close the aperture of the shell when the animal contracts into the shell. The operculum is found generally among marine snails and in all fresh-water snails that breathe by means of a gill. It is lacking in land and all fresh-water snails that have a pulmonary sac.

Draw a snail with its shell from the side.

5. **Mantle.**—The mantle is a fold of the body wall which lines the inside of the body whorl where it can readily be seen in living or preserved specimens as a fleshy layer. It is inseparably fastened in life to the inner surface of the shell. Study the mantle in a preserved snail, gently pulling the snail out from the shell (in life the animal cannot leave the shell but after death the mantle separates from the shell). The mantle is seen as a cone-shaped fold which covers the dorsal part of the animal. Its ventral thickened muscular margin, called the *collar,* is free from the body in gill-breathing snails but is fastened to it in lung-breathing forms. The cavity between the mantle and the body is called the *mantle* or *pallial cavity* and serves chiefly for respiratory purposes. In many marine snails the collar of the mantle is drawn out on the left side into a spoutlike process, the *siphon,* which in life fits into the siphonal canal of the shell and serves to conduct a current of water into the mantle cavity.

6. **Visceral mass.**—The viscera are contained chiefly in the dorsal part of the body which as seen in a snail removed from its shell is spirally coiled corresponding to the coils of the shell. This spiral mass of viscera is called the *visceral mass* or *visceral coil.* It is closely covered externally by the body wall, very thin here, from which the mantle projects ventrally. The dissection of the visceral mass will not be attempted. Suffice it to say that it contains the greater part of the digestive tract including a large digestive gland ("liver"), the nephridium or kidney, the reproductive organs, and the heart inclosed in a pericardial chamber. The snail thus has well-developed systems of

organs. The visceral mass is solid, a coelome being represented only by the pericardial cavity.

Draw from the side a snail removed from its shell.

7. Respiratory system.—The organs of respiration consist of a *gill* (sometimes two) in nearly all marine forms and in those fresh-water forms which possess an operculum, or of the mantle wall (so-called *pulmonary sac* or *lung*) in non-operculate fresh-water snails and land snails. Slit open the mantle along its mid-dorsal line. Note the large mantle cavity within. On the left (snail's left) half of the mantle wall look for the *gill* in gill-breathing forms. It usually consists of parallel plates fastened to the mantle wall. Below it may be a smaller gill-like structure, the *osphradium,* believed to be an organ of chemical sense. In the case of lung-breathing (*pulmonate*) snails, the inner surface of the mantle wall is seen permeated with a rich network of blood vessels. The mantle chamber of pulmonate snails thus serves as a *lung*. It opens to the exterior by an opening, the *pneumostome,* generally at the right side, in some cases on the left side, usually situated on a conical papilla. In the right mantle wall may be noted the terminal part of the intestine, opening by the anus near the pneumostome. The genital opening may be near the anal opening but in pulmonate snails is generally on the side of the head below the right tentacle.

8. Radula.—The radula is an organ peculiar to the Mollusca. Slit open the body wall in the mid-dorsal line of the head from the mouth to a point between the tentacles. Find in the interior a stout muscular rounded mass, the *buccal mass*. Open this and note in its dorsal part in pulmonate snails a hardened curved *jaw* (two in some pulmonates). Below this are fleshy projections. Tease the central one and try to find in it a brownish hardened structure, the *radula*. Remove it and examine under low power of the microscope. Note numerous *teeth* upon its surface. In snails with a proboscis the radula is a long ribbon-like structure in the proboscis. The radula is worked by muscles back and forth over the end of cartilages like a rope over a pulley, producing a rasping action on the food.

9. Ecology of the snail.—The common pulmonate pond snails may readily be kept in laboratories in jars of water containing vegetation. The common forms are: *Lymnaea,* with a long spiral shell; *Planorbis,* with a flat spiral shell; and *Physa,* short spiral shell, abruptly enlarging to form the body whorl.

a) *General activities and locomotion.* What are the snails doing in the jar? Observe use of the foot in crawling. Is the crawling accomplished by muscular action? Can you see muscular waves passing along the foot? Can snails swim free in the water? How do they reach the surface of the water? Can they travel up and down in the water without climbing on objects? Consider here application of Archimedes' law (a body immersed in a liquid loses as much in weight as the weight of the liquid it displaces). How could a snail increase its surface so as to decrease its weight? Remove a snail from the water and throw it back forcibly into the water. Does it sink? Does it let out bubbles of air? These pond snails do in fact possess a chamber filled with air. Explain how this would help the snail to rise and sink in the water.

Observe the mud in the bottom of the jars where snails are kept, or provide such a layer of mud. Note the trails left by the snails as they crawl. Stir up the trail and note

that the particles stick together. Explain. Is this habit of the snail of advantage in crawling over soft mud or slippery surfaces? By leaving a similar trail in the water could the snail produce the appearance of moving about free in the water? If you see a snail apparently suspended in the water, pass an instrument around the snail and see if it is actually clinging to a *mucus thread.*

Remove a snail from the water. What does it do? Is this reaction of advantage? What then is the advantage of the shell? What disadvantages? Poke the snail rather strongly and see how far it can withdraw into its shell.

b) *Food taking:* As the animal crawls along the side of a glass jar note a wedge-shaped structure protruded at regular intervals from the mouth. This is the tip of the buccal mass, described above, and its movements serve to bring the radula in contact with the food. What does a snail get in the way of food by rasping the glass? Do the snails feed on the plants in the aquarium? What might they get besides plant tissues by scraping the surface of plants? Examine scrapings from the glass and plants under the microscope. What do you see (*A*)? Place a piece of fresh raw meat in the aquarium and observe behavior of the snails. Do they detect the food before coming in contact with it?

c) *Respiration:* Watch the snails for some time and observe that eventually they come to the surface and assume a certain attitude. (How can a snail heavier than water keep on the surface?) Observe a small conical projection at one side between body and shell. This bears the pneumostome. Note that the tip of this projection is thrust above the surface film and the pneumostome opened. How long does it remain open?

What stimulus do you suppose drives the snail to the surface for air? What factors would determine how often it would need to come to the surface? Try difference in number of visits to the surface for air between snails kept at ordinary temperatures and those kept in ice water. How does the snail "know" in which direction to go for air? To determine this, take a bottle, put several snails in it, fill completely with water, stopper tightly so that all air bubbles are excluded, turn upside down, and note in which direction the snails go for air. Does this answer the question?

Determine how long snails will live without air. Take two bottles of equal size and place an equal number of snails of the same size in each. Fill the bottles completely with water and stopper tightly. Place one in the ice-box and leave the other at room temperature. How long do the snails survive in the stoppered bottles? Does temperature make a difference? Why? Does the experiment show that pulmonate snails can use the oxygen dissolved in the water? How do the results of this experiment apply to the living conditions of snails in winter, when the ponds may be covered with ice and snails cannot get to the surface?

d) *Desiccation experiment:* Take a glass dish, put about an inch of water and several snails in it. Let stand open to the air until the water dries up. What do the snails do? Can they carry on activities in the absence of water? Pick one up and examine. What do you find across the mouth of the shell? Are the snails dead? Put some water in the dish and note results. How does this behavior to dryness apply to the possibility of ponds drying up in hot weather?

e) *Reaction to light:* Distribute a number of snails equally over the bottom of a large pan or vessel of water, set in the light, and darken one half. Note after a time the distribution of the snails with respect to the light.

f) *Reproduction:* The gelatinous egg masses of snails will commonly be found in jars where snails are kept. Where are they placed by the snails? Why are they not dropped on the bottom? Observe the process of development by removing eggs from time to time and studying under the microscope. Are there any free larval stages? Are the young at hatching like the adults?

B. ANATOMY OF A CLÀM

The clams and mussels are common animals of fresh and salt waters, distinguished by the *shell* composed of two halves or *valves* in which they are inclosed. On this account these animals are commonly designated as *bivalve mollusks.*

1. **General features.**—Examine a preserved specimen. The animal is wholly or almost wholly inclosed in a bivalved shell, which constitutes its *exoskeleton.* The region where the two valves are united is *dorsal,* the opposite region *ventral.* Dorsally each valve presents a swollen projection, the *umbo,* which is usually nearer the *anterior* than the *posterior* end. In many marine bivalves, one or two tubes, the *siphons,* project from the posterior end. Dorsal, ventral, anterior, and posterior ends being thus defined, the valves are seen to cover the right and left sides of the animal. Is the animal bilaterally symmetrical? Do you see any evidence of a head?

2. **Exoskeleton or shell.**—Examine empty valves of various bivalves. Study the external surface first. The dorsal margin of each valve is hinged to its fellow by a tough elastic band, called the *hinge ligament.* This ligament causes the valves to open so that empty shells always gape. The umbo was noted above. The surface of the shell is marked by curved lines, the *lines of growth,* which are arranged concentrically with regard to the umbo. The umbo is the oldest part of the shell and growth occurs by additions at the free margins of the valves. **Draw** the shell from external view.

Examine the internal surface. It is smooth, pearly, and concave. It is marked by various *impressions* caused by the attachment to it of various parts of the animal. Parallel to the free margin runs a line, the *pallial line,* to which in the intact animal the mantle (described later) is attached. At the posterior end of the shell in those bivalves which possess siphon tubes, the pallial line is bent inward, forming the *pallial sinus,* caused by the attachment there of the muscles which operate the siphon tubes. The pallial line ends on each side in a large oval impression; these are the *scars* of the *anterior* and *posterior adductor muscles,* the powerful muscles which close the valves. Smaller scars, frequently not very distinct, occur in contact with the adductor scars; in freshwater bivalves there are two of these back of the anterior adductor scar and one above the posterior adductor scar.

Near the dorsal margin below the hinge are found in most shells (absent in some) projections known as *teeth,* which are commonly of two kinds: short projections, the *cardinal* teeth, and long low ridges, the *lateral* teeth. Is the arrangement of the teeth the same on right and left valves? Why? **Draw** a valve from the inner surface, stating whether it is a right or left valve.

The shell consists of an outer thin layer, the *periostracum,* generally brown in color, of organic nature, often worn off in spots, and of a thick inner white *calcareous* layer, composed of calcium carbonate. The hinge ligament is composed of periostracum.

3. Mantle and siphons.—Return to the preserved specimen. Pry the ventral margins of the valves apart and insert an object between them to keep them apart. Look into the interior. A membrane, the *mantle,* is closely adherent to the inner surface of each valve. Between the two mantle lobes is seen the body of the animal. Free the mantle from one valve by carefully passing a scalpel between the mantle and the inner surface of the valve. If the valves are sufficiently open, the adductor muscles may be seen holding the valves together. If you cannot see them, feel for them with the scalpel in the places where you saw the muscle scars in studying the shell. Cut through the adductor muscles, at their attachment to the valve from which the mantle was just freed and remove this valve, leaving the body of the animal in the other.

The mantle is now seen to consist of two somewhat oval *lobes,* which inclose between them the remaining structures of the animal. The large cavity between the two mantle lobes is called the *mantle* or *pallial chamber.* The mantle lobes are simply outgrowths from the dorsal region of the animal. Note line on each mantle lobe where it attaches to the pallial line of the shell. The ventral border of the mantle from this attachment to its free margin is thickened and in fact contains considerable muscle tissue which in life serves to protrude or retract the mantle borders as the valves are opened or closed, respectively. In some bivalves (not in fresh-water forms) the ventral margins of the mantle lobes are more or less fused together. The mantle secretes the shell, forming periostracum only at its free margin but adding continuously to the calcareous layer over its whole surface so that this layer becomes thicker with age.

At the posterior end of the animal, the mantle lobes form the *siphons.* These are two openings for the intake and egress of water. They differ considerably in different bivalves. In all of the larger fresh-water bivalves, the siphons are formed simply by the contact (without fusion) of the margins of the mantle lobes. The more dorsal and smaller opening is the *exhalant* or *excurrent* siphon, serving for the exit of water; the ventral and larger opening is the *inhalant* or *incurrent* siphon, used for the intake of water. Both openings have dark-colored *lips,* those of the incurrent siphon being beset with *papillae* probably of sensory function.

In many marine clams, the siphons are extended into shorter or longer tubes, sometimes very long and highly muscular, separate or fused for part or the whole of their length. Whenever the siphons take the form of tubes, the muscular border of the mantle lobes is greatly strengthened at the base of the siphons forming the *retractors of the siphons,* by means of which the siphons can be drawn in. The attachment of these retractors to the shell causes the pallial sinus noted above.

Note muscle masses projecting through the mantle at anterior and posterior ends of the animal. The largest of these are the *anterior* and *posterior adductors,* which serve to close the valves.

4. General internal features.—Cut away the loose parts of the mantle lobe that you freed from the shell. The body of the animal is now apparent as a soft elongated mass, flattened laterally, and ending ventrally in a keel-like structure, the *foot.* The

foot is hard and firm owing to its muscularity. The softer stouter part of the animal dorsal to the foot is the *visceral mass,* which contains the internal organs. Partly covering the visceral mass on each side are two long thin flaps, the *gills* or respiratory organs. In some specimens (fresh-water clams only), all or part of the gills may be greatly thickened owing to the presence of developing embryos inside. The most dorsal part of the animal dorsal to the gills consists of a thin membrane inclosing a large cavity. This cavity is the *pericardial cavity*. Care should be taken not to injure it.

The anterior end of the animal is the end opposite the siphons. Is a head present? Note that the foot is directed toward the anterior end. Near the anterior end are seen on each side two flaps, the *labial palps*. Their posterior ends are free, their anterior ends united to each other and to the mantle. Follow the two palps of one side anteriorly. They are seen to narrow to mere folds, the *upper* and *lower lips,* and between these in the median line, directly behind the anterior adductor muscle in fresh-water clams, is found an elongated slit, the *mouth*. By way of the lips the two palps of one side are continuous with those of the other. The palps act to direct food into the mouth. The whole inner surface of the mantle, the gills, and the palps are heavily ciliated, causing a current of water to circulate throughout the mantle chamber. Microscopic food material in the water is collected on the labial palps by means of rather complicated ciliary currents and then proceeds down the groove between the palps into the mouth.

Draw the animal from the side.

5. Circulatory system.—The *pericardial cavity* is the large cavity situated in the posterior part of the dorsal region of the animal. It represents nearly all that remains of the extensive *coelome* found in more primitive mollusks. Carefully remove one thin wall of the pericardial cavity. A tube, the terminal part of the intestine, is seen passing through the cavity. Wrapped around the central part of this tube, is a spongy structure, the *ventricle* of the *heart*. From the ventricle a very thin-walled, fan-shaped *auricle* extends downward on each side. The auricles are best seen by carefully raising the intestine so that the heart is put on a stretch. The heart of bivalves thus consists of one ventricle and two auricles. The broadest part of each auricle is attached to the dorsal border of the adjacent gills from which the auricle receives aerated blood. From the anterior end of the ventricle a vessel, the *anterior aorta,* is given off along the dorsal surface of the intestine; and from the posterior end of the ventricle a *ventral aorta* passes backward along the under surface of the intestine. These two aortae are often difficult to see. The remainder of the circulatory system is impractical to dissect (see texts). Note that the circulatory system of mollusks is of the *open* type, that is, there are no definite veins but the circulation is completed through body spaces, called *sinuses*.

Draw pericardial cavity and its contents.

6. Respiratory system.—This consists of the gills which appear as two plates (*laminae*) on each side of the visceral mass. These two laminae really constitute one gill so that the animal possesses but a single pair of gills. Observe that the gill is marked by stripes running parallel from dorsal to ventral border. These stripes represent partitions which divide the interior of the gill up into vertical tubes, the *water tubes*. Make a cut across a lamina at right angles to the partitions and note on the cut

surface the cross-sections of the water tubes. Study the attachments of the dorsal borders of both laminae of the gills. Are they attached to the mantle? visceral mass? Break through the dorsal border of the outer lamina where it is continuous with the mantle lobe and find within it a canal, the *suprabranchial chamber,* which runs the length of the gill. Trace this canal posteriorly and note that it opens into a wide chamber below the posterior adductor muscle. Determine if there is a suprabranchial chamber in the dorsal border of the inner gill lamina. Now turn the animal with posterior end toward you and look into the exhalant siphon (not possible on bivalves which possess siphon tubes). Observe that the four gill laminae are united behind the visceral mass and are also united at this point with the mantle forming a partition between the exhalant and inhalant siphons. Note further that the four suprabranchial chambers come together into one chamber below the posterior adductor muscle and that this chamber leads directly to the exhalant siphon. Further details of the structure of the gills will be found in texts.

This respiratory system operates in the following manner. There are microscopic openings on the surfaces of the gill laminae. Through these a water current is driven by ciliary action into the water tubes. The current then passes dorsally in the water tubes to the suprabranchial chambers and along them to and out of the exhalant siphon. A current of water thus circulates in the interior of the gills. Exchange of respiratory gases occurs through the walls of blood spaces located in the partitions between the water tubes.

As already stated, the females of fresh-water clams brood their young in the water tubes of the gills. Certain parts of the gills of some specimens may, therefore, be found stuffed full of small objects which are embryonic clams.

7. Excretory system.—This consists chiefly of a pair of *nephridia* located in the floor of the pericardial cavity. Remove the gills from the side on which you have been dissecting and expose the pericardial cavity fully. In the floor of the cavity some brownish structures can be dimly seen; these are the nephridia. They open into the floor of the pericardial cavity at its anterior end by a pair of small pores, the *reno-pericardial openings,* which can usually be seen in large specimens. Break through the floor of the pericardial cavity gently with a probe. You should find a pair of tubes composed of a soft brownish material running the length of the pericardial chamber. These constitute the greater part of the nephridia but their exact course is difficult to determine and should be learned from textbooks. Each nephridium opens to the exterior by a minute pore difficult to find, located at the anterior end of the inner gill lamina in the angle between the lamina and the visceral mass.

8. Nervous system.—In correlation with the absence of a definite head and the sedentary habits of the bivalve mollusks, their nervous systems are considerably reduced in comparison with those of other mollusks, as snails. The nervous system consists of three pairs of ganglia, connected by commissures: the *cerebro-pleural, pedal,* and *visceral ganglia.* The two cerebro-pleural ganglia are separate from each other. One occurs on each side of the mouth in the upper lip fold. On picking away the surface tissue at this place the ganglion is revealed as a small yellow or brownish branching body. It is connected to its fellow on the opposite side by the *cerebral commissure*

passing in the upper lip above the mouth, not practical to find. The visceral ganglia are united into a large star-shaped mass located on the ventral surface of the posterior adductor muscle. Locate it there and remove the thin membrane which covers it. Note nerves extending from it to adjacent parts. Cerebro-pleural and visceral ganglia are connected by commissures not practical to find. The pedal ganglia are also united to one mass located in the anterior part of the foot. To find it split the foot in the exact median line and carry the cut dorsally until you strike the soft tissues of the visceral mass. Look for a brownish bilobed mass in the anterior part of the boundary between foot and visceral mass. The pedal ganglia are connected with the cerebro-pleural by commissures and a circle around the beginning of the digestive tract is thus formed similar to the arrangement in the earthworm.

9. Muscular system.—The muscles of the mantle border, siphons, and the adductors of the shell have already been noted. Note muscle fibers just under the surface membrane of the visceral mass. These fibers converge on each side to a stalk which extends posteriorly to a position just above that of the posterior adductor muscle. This pair of muscles is named the *posterior retractors of the foot*. Observe a similar pair of *anterior retractors* extending forward and dorsally to a position above the mouth. Just in front of each pair of labial palps is seen (in fresh-water clams) still another muscle on each side, the *protractors* of the foot. These extend the foot by compression of the visceral mass; the retractors draw it back in. These muscles leave small scars on the valves near the adductor scars.

10. Digestive system.—The labial palps and mouth were already noted. The posterior part of the intestine has already been seen as it passes through the pericardial chamber. Trace it now posteriorly and find the *anal opening* on the dorsal surface of the posterior adductor muscle. The remainder of the digestive tract is imbedded in the visceral mass and is almost impossible to dissect out as the visceral mass is filled with connective and muscle tissue. The simplest method of revealing the digestive tract is to bisect the visceral mass. Remove the animal from the valve and bisect the visceral mass from ventral to dorsal side. In the cut surface the digestive tract appears. Note that the mouth leads into an enlarged cavity, the *stomach,* surrounded by a pair of greenish *digestive glands*. From the stomach the *intestine* leads into the visceral mass where it makes a loop. It then passes to the right of the loop and proceeds dorsally where it emerges into the pericardial cavity. The remainder of its course was already seen.

Draw the section showing the digestive tract. **Indicate** on this drawing the positions of the nerve ganglia.

11. Reproductive system.—The sexes are separate in the great majority of the bivalves. Externally in some fresh-water forms females can be distinguished from males by the greater convexity of their valves. The reproductive organs consist of a pair of yellowish branched structures which are diffused throughout the visceral mass where they can be seen between the coils of the intestine in the section made above. Each gonad opens by a minute pore near the external opening of the kidneys. It is generally impractical to find these openings.

Indicate reproductive organ on the drawing made above.

12. Development of fresh-water clams.—In fresh-water bivalves as already noted, the young develop in the water tubes of the gills to a certain stage. All four gill laminae may serve as *brood pouches*, or only the outer lamina, or only the posterior half of the outer lamina. If specimens with young are available remove some young from the gill and examine under the low power of the microscope. The young are known as *glochidia*. Each glochidium is a small bivalved object, with a mantle lobe lining each valve and a single adductor muscle extending between the valves. The glochidia are discharged from the clam in the outgoing water current. They then fasten themselves to the gills of some passing fish by clapping their valves over the gill filaments of the fish. In the gills of the fish they live as parasites while transforming into miniature clams. When this transformation is complete they drop off from the fish and take up the usual mode of life.

Draw a glochidium.

13. Behavior of living clams.—Observations on living specimens will best illustrate how the clam uses the structures we have seen. Clams occur in the bottoms of ponds and rivers wherever such bottoms are composed of firm materials such as sand and gravel. They cannot live in muddy bottoms for reasons that will be shortly apparent. Build up a sand bank in an aquarium full of water and place a live clam on the sand bank. Observe behavior of the clam. Note opening of the valves and protrusion of the foot. Understand how these movements are accomplished by the muscles which were studied. Can you see the ventral borders of the mantle lobes? How are they used? Observe how the animal buries itself in the sand. Do you think it could accomplish this in a muddy bottom? What position does the clam assume when buried? Which end is buried? Which protruded? Why? Observe the appearance of the siphons. Touch them. What happens? Which of the two siphons is the more sensitive? Prove that there are water currents at the siphons by adding a little powdered carmine or carbon suspension to the water near the siphons. Make a diagram in your notebook showing the course of the water currents. From these observations of the habits of clams do you understand the poor development of head in these animals? Write up an account of your observations on the behavior of the clam in your notebook.

Cut out small bits of gills, palps and mantle from a living clam that has been opened and examine them in water under the microscope. Note cilia on these structures.

14. General considerations on mollusks.—What type of symmetry is exhibited by Mollusca? Do they show any signs of segmentation? How do you think the head of such mollusks as the snail compares with the head of a flatworm, an annelid, or the frog? Do the habits of the clam account for its lack of head? What other animals have we studied in which the anterior end was poorly developed in correlation with sedentary habits? What is the advantage of the shell of Mollusca? Is it disadvantageous in any way? Do you think the foot is a very efficient organ of locomotion? Do the mollusks have all of the systems of organs that we found in the frog? Do they have any that were lacking in the flatworm? starfish? annelid? What system seemed to you particularly well-developed in the clam? What differences exist between the circulatory systems of the clam and earthworm? Is the clam a triploblastic animal? Is it

coelomate? State what structures of the clam seemed to you to adapt it for its particular mode of life.

C. GENERAL SURVEY OF MOLLUSCA

1. Class Amphineura.—These are the most primitive mollusks. They are commonly known as chitons and are fairly common animals of the rocky shores of the sea. They are somewhat similar to snails in appearance. The dorsal surface carries a shell composed of eight separate valves. The ventral surface consists chiefly of the broad foot in front of which is a well-developed head. Foot and head are encircled by a fold which is the free border of the mantle. Between the mantle and the body is a circlet of gill filaments.

2. Class Pelecypoda.—This class includes all of the bivalve mollusca. Different forms and shapes of shells should be examined. In the oysters one shell is cemented to a rock and the foot is degenerate. The scallops or pectens have symmetrical fluted shells. They are able to swim about by clapping the valves together. They possess another interesting feature in the presence of a row of eyes along the free edge of each mantle lobe. Many marine bivalves are provided with exceedingly long stout siphon tubes; such forms lie buried deeply in the bottom with only the tips of the siphons protruding at the surface.

3. Class Gasteropoda.—This class includes the univalve mollusks, commonly known as snails. Various types of shells may be examined. The limpets have simple cone-shaped shells. In some forms there are holes in the shell; among the best known of these is the abalone of the Pacific Coast in which there is a row of holes in the shell. These holes are used for the exit of water. The slugs are land snails in which a shell is lacking or is reduced and inclosed in the mantle. The nudibranchs are highly colored marine snails which lack a shell but possess gills on the dorsal surface of the body. In some snails the mantle is so highly developed that it partly or wholly incloses the shell. In others the foot is expanded into winglike processes by which the animal can swim about.

4. Class Cephalopoda.—In these animals the foot is modified into a set of armlike processes bearing numerous suckers. These arms are used in the capture of prey. Among the best known members of this class are the squids with torpedo-shaped bodies, the octopuses or devil-fish with saclike bodies and long snaky arms, and the chambered Nautilus which inhabits a spirally coiled shell, divided into chambers by partitions. The cephalopods have well-developed heads and brains and large eyes constructed much like the eyes of vertebrates. They are among the most highly organized of all the invertebrates.

XIV. PHYLUM ARTHROPODA

This phylum, the largest in the animal kingdom, is at once recognized by the presence of *jointed appendages* attached to a more or less segmented body. The arthropods are *triploblastic* animals, with a *reduced coelome*, the body spaces being of the nature of *blood sinuses*, and with well-developed systems of organs. This phylum will be illustrated by the study of two members belonging to different classes of the phylum.

A. ANATOMY OF THE LOBSTER (OR CRAYFISH)

The lobster or crayfish are commonly employed as representatives of the class Crustacea, a group of arthropods distinguished by the possession of a hard exoskeleton. Either animal may be used, the lobster being preferable because of its greater size. The slight differences between the two forms will be noted in the course of the description.

1. External anatomy.—Examine a preserved specimen. The animal has a hard external covering, the *exoskeleton*, which corresponds to the cuticle of the earthworm and the shells of mollusks and like them is a secretion of the ectoderm. The exoskeleton is composed of an organic substance called *chitin*, hardened by the deposit in it of calcium carbonate. Identify anterior and posterior ends, dorsal and ventral surfaces. Is the animal bilaterally symmetrical? Is it segmented? Is it clearly segmented along the whole axis of the body, like the annelids? What part of the body is most evidently segmented? what least?

The body differs greatly from the other invertebrates studied and resembles the frog in that it is divided into definite regions, the *head*, *thorax*, and *abdomen*. Head and thorax are, however, more or less fused into one region, called the *cephalothorax*. The single piece of the exoskeleton which covers the cephalothorax dorsally and laterally is called the *carapace*. A groove runs across the mid-dorsal region of the carapace and obliquely forward on either side. This is the *cervical groove* and separates the head in front from the thorax behind. Segmentation has been lost on the dorsal side of the cephalothorax through fusion of segments.

Another striking difference between the lobster and the other invertebrates previously studied is the presence of *jointed appendages*. Each segment of the body is represented by a pair of appendages, and it is thus possible to determine the number of segments even where the lines between them have been lost by fusion. In many arthropods, however, some of the appendages have been lost also, so that the determination of the number of segments in the body is sometimes a matter of great difficulty.

a) *The head:* The head terminates anteriorly in a spiny pointed projection of the carapace, the *rostrum*. The head is provided with a number of sense organs, exceeding, in variety and complexity, those of the lower invertebrates. There is a pair of large, stalked, movable *eyes*, which are probably not appendages. In front of the eyes occurs the first pair of appendages, the *antennules*, short, forked filamentous outgrowths. Just below these is the second pair of appendages, the *antennae*, long, flexible, many-jointed structures. Both antennae and antennules have tactile and chemical functions. On the

ventral side of the head are additional appendages belonging to the head, surrounding the mouth and used for tasting, handling, and tearing food. These will be studied later.

b) The thorax: Two longitudinal lines on the dorsal surface of the thorax divide the carapace into a median *cardiac* region, covering the heart, and two broad, curved, lateral *branchial* regions, which cover the chamber containing the gills. The ventral side of the thorax bears five pairs of walking legs, of which the anterior pair is modified into large pincers; some of the appendages in front of the pincers also belong to the thorax.

c) The abdomen: The abdomen is plainly divided into seven joints or segments, six of which bear appendages, used for swimming. The first pair of abdominal appendages in male and female lobsters and in female crayfishes and the first two pairs in male crayfishes are modified for sexual purposes. The last pair of abdominal appendages is greatly broadened and forms with the last abdominal segment a broad *swimming fan*. The last segment, or *telson*, has no appendages and bears the anus on its median ventral surface. There is some doubt that the telson is a true segment.

d) Study of a typical segment and pair of appendages: As the abdominal segments are more distinct and less modified than the other segments, one of them, as the third or fourth, may be selected for study. Such a segment has the general shape of a ring, as in the annelids. Its exoskeletal covering may be divided into a convex dorsal portion, the *tergum*, a thin lateral plate, the *pleuron*, extending free ventrally into a point, and a slender ventral bar, the *sternum*. Between successive segments occur thin *arthropodial membranes*, where the calcareous deposit is lacking; these are best seen on the ventral surface between successive sterna, and they permit movement of the segments upon one another. Examine the joints between segments at the pleura; determine how they work by bending and straightening the abdomen.

Between the base of the pleuron and the sternum of the segment is a round area, into which the appendage is fastened by means of an arthropodial membrane. Cut through this membrane and remove a complete appendage. It has the following parts: It springs from the segment by a basal stem, the *protopod*. This really consists of two joints, a very small basal piece, the *coxopod*, and a long distal part, the *basipod*. From the basipod arise two flattened, many-jointed plates. The outer of these is the *exopod*; the inner one, next the median line, the *endopod*. Make a diagram of an ideal cross-section of the segment with its pair of appendages.

This type of appendage found on the abdominal segments is called the two-forked or *biramous* appendage. It is supposed to be the primitive arthropod appendage, all other kinds of appendages found in arthropods being derived from it by modification. Theoretically, the original arthropod consisted of a series of segments, like the abdominal segments of the lobster, all bearing biramous appendages.

e) Comparative study of the appendages: The appendages will be studied by comparing them with the typical biramous appendage described in the preceding section, and determining what modifications have occurred.

(1) The abdominal appendages: These are designated as *swimmerets*, or *pleopods*, since they are employed in swimming. In the female they also serve as places of attachment of the eggs while developing. The swimmerets of the second to fifth abdom-

inal segments are very similar to the typical appendage already described. The sixth pair of swimmerets, called the *uropods*, is greatly enlarged and forms with the telson a powerful swimming organ, the *tail fan*. Determine other differences between the uropods and the other swimmerets. The first pair of swimmerets in the female lobster and crayfish is greatly reduced but otherwise similar to the others. The first pair of swimmerets in the male lobster and the first and second pairs in the male crayfish are modified for the transference of the sperm to the female.

(2) The thoracic appendages: Remove the branchial region of the carapace from the *left* side by lifting it up and cutting away the free portion. The *branchial chamber* is thus exposed. It is filled with a number of feathery *gills*, which are the respiratory organs. Observe that all of the thoracic appendages have gill-bearing processes extending up into the branchial chamber. The thoracic appendages comprise five pairs of walking legs, called *pereiopods*, and three pairs of smaller appendages, called *maxillipeds*, anterior to them.

In removing appendages first break through the arthropodial membrane completely around the attached end of the appendage and gently pull the appendage off. Remove in this way the left *third maxilliped*, the appendage just in front of the large pincers. Be sure to get the gill-bearing process with it. The basal joint, the coxopod, of the third maxilliped bears a delicate, hairy, leaflike expansion, the *epipod*, to which a feathery *gill* is attached. Notice the great freedom of movement of the coxopod and explain. The next joint distal to the coxopod is the basipod. From it arise two branches, an inner endopod, consisting of five joints, and an outer exopod, of many small joints. The maxilliped is therefore a biramous appendage, similar to the swimmeret, but showing a process of reduction of the exopod. The basal joint of the endopod has a row of hard teeth, used in crushing food.

Remove the left second pereiopod with all of its parts and compare with the third maxilliped, placing both before you in the same position. The pereiopod has a coxopod with an epipod and gill, a basipod, and an endopod of five joints. The comparison shows, however, that the exopod is completely lacking, and the pereiopod is therefore a *uniramous* appendage. Observe the pincer at the end of the pereiopod and determine how it is formed.

Examine the other pereiopods, comparing them with the second one and noting differences. Do all have pincers, epipods, gills? By moving the legs, note how the gills are moved. In what directions can the legs be moved upon the body? In what directions can the joints of the leg be moved upon each other?

The first pair of pereiopods is greatly enlarged, with a powerful pincer, or *chela*. Note that the two chelae of the lobster are not alike, but one is massive with broad crushing surfaces, called the *cracker claw*, and the other sharper and more slender, with little teeth, known as the *toothed claw*.

On the inner side of the coxopod of the third pereiopods of the female find the *female genital openings*. The *male genital* openings are in the same place on the fifth pereiopods.

Draw the third maxilliped and the second pereiopod in the same positions.

Next remove the left *second* maxilliped complete. Compare with the third maxil-

liped. Does it have the same parts? Notice reduction of the epipod and gill and the presence of only four joints in the endopod.

Remove the *first* maxilliped, and compare with the other two. Does it have an epipod? gill? Observe particularly that the appendage has become more broadened and leaflike. This is due to the moving of the coxopod and basipod from their original basal position to form the medial side of the appendage. The endopod is thus shoved laterally. Endopod and exopod of the first maxilliped are slender processes of about equal size, the exopod resting in a groove of the endopod. Endopod is thus gradually being reduced, consisting now of but two segments, and the two segments of the protopod are being gradually broadened and shifted to the inside. There is thus produced the *foliaceous* type of appendage.

The thorax therefore has eight pairs of appendages, the three pairs of maxillipeds and the five pairs of pereiopods, and consists of eight segments.

(3) The head appendages: The head has five pairs of appendages, omitting the eyes. These are, beginning with the most posterior, two pairs of *maxillae*, a pair of *mandibles*, the *antennae*, and the *antennules*.

Examine the *second* maxilla in place. It is the appendage in front of the first maxilliped. Does it have an epipod? gill? Observe that the epipod is continuous with an anterior process, which is the exopod, the whole forming an elongated blade, pointed at both ends, which fits into the anterior end of the branchial chamber. This blade, the *bailer* or *scaphognathite*, moves back and forth, drawing a current of water through the branchial chamber over the gills from the posterior end of the chamber forward. Medial to the exopod is a slender endopod, still further reduced and consisting of but one joint, and medial to this the expanded protopod, with four processes. The second maxilla is thus decidedly foliaceous. Remove it and draw.

The *first* maxilla is a reduced foliaceous appendage. Its two inner thin plates are the protopod; the outer slender process is the endopod, exopod being absent. Remove the first maxilla. The small process in front of it is not considered to be an appendage but is a part of the lower lip.

The heavy mandible is now exposed. It consists of a single triangular piece with strong teeth upon its inner edge; and a small palp, probably the endopod, folded beneath the toothed margin. Spread the mandibles apart so as to see the mouth opening. In front of the mouth opening is the cushion-like upper lip, or *labrum*.

Examine the antenna. In the middle of the ventral surface of its basal segment (coxopod) find the *renal opening*, the opening of the excretory organ. The long many-jointed filament is the endopod; the thin sharp projection near the base of the filament is the exopod. Draw the antenna.

The antennule or first antenna has a protopod of three joints from which arise two short many-jointed filaments, which are probably exopod and endopod.

This investigation of the appendages shows that there are at least five segments to the head, eight to the thorax, and six to the abdomen, or nineteen appendage-bearing segments. If the telson is a segment, as seems reasonable, the lobster consists of twenty segments. Some zoölogists believe that the eyes also represent a segment and raise the number to twenty-one, but considering that eyes occur on unsegmented animals it

seems probable that they are not a pair of appendages homologous to the others.

f) The respiratory system and the branchial chamber: Study the arrangement of the *gills*, or *branchiae*, the respiratory organs, in the left branchial chamber, where they have already been exposed. We have noted that one gill is fastened to the epipod of most of the thoracic appendages. Such gills fastened to appendages are called *podobranchiae*. Remove the podobranchia and epipod from the third pereiopod, and observe that two more gills are situated internal to them attached to the arthropodial membrane. These are *arthrobranchiae*. In the lobster there is still a third set of gills, called the *pleurobranchiae*, found attached to the thoracic walls when the arthrobranchiae are removed. Each thoracic segment has, therefore, typically four gills, but not all of them possess the full number, as the student may readily discover. The crayfish has no pleurobranchiae.

Cut off one of the gills and examine its structure. It consists of a central axis bearing numerous delicate threadlike filaments. Examine the cut surface of the axis and note the two canals which it contains, one for blood to enter the filaments, the *afferent* vessel, and one for it to leave the filaments, the *efferent* vessel. Note the hole left in the thoracic wall where the gill was removed through which the blood vessels pass.

Remove all the gills from the branchial chamber and note that the segmentation of the thorax is now visible. Examine the region where the extension of the carapace over the branchial chamber was cut off and see that this extension was not really the sides of the thorax (which are concealed by the gills) but merely a downward fold from the median cardiac region.

g) The special sense organs: These are best studied before the animal is dissected. The body and appendages of the lobster bear innumerable hairs, many of which are sensory hairs attached to nerve cells and having tactile functions. Many hairs upon the antennules, antennae, and mouth appendages are also organs of taste and general chemical sense. The head appendages are more sensitive than the others.

The eyes of the lobster are compound eyes, i.e., they are made up of a large number of simple eyes, or *ommatidia*, which are radially arranged. The cuticle covering the eye is thin, transparent, and flexible, lacking the calcareous deposit. It is called the *cornea*. Examine it with the hand lens, noting the minute polygonal areas of which it is composed. Each of these is the outer end of one of the ommatidia. Make a longitudinal section through the eye and eye stalk with a sharp knife and examine the cut surface. Observe the numerous black ommatidia radiating from a central white region, which is the *optic ganglion*. See books for structure of an ommatidum. Then remove some of the ommatidia from the section of the eye, tease on a slide in a drop of water, cover, and examine. You should be able to identify the *crystalline cone*, the *rhabdome* surrounded by black *pigment cells*, and the nerve fiber leading away from each ommatidium.

The *statocyst*, or *organ of equilibrium*, is a thin sac located in the basal segment of the antennules. Remove an antennule and cut off the ventral wall of the basal segment of the antennule. This reveals a thin-walled sac containing sand grains, attached to the dorsal wall of the segment. The assistant should demonstrate the crescent of *sensory hairs* within the sac.

2. **Internal anatomy and systems.**—With a scissors carefully remove piece by piece the entire dorsal half of the exoskeleton from rostrum to telson, beginning at the anterior end. In doing this certain structures are to be observed so that the next two sections should be read while cutting. Be careful not to injure blood vessels which will probably be injected with a yellow fluid.

a) *Integumentary system:* As already noted the exoskeleton or shell is secreted by the underlying ectoderm and is thus part of the integumentary system. In removing the exoskeleton, the ectoderm, a thin membrane called the *epidermis* (or *hypodermis*), will be noted adhering to the inner surface of the exoskeleton. The Crustacea shed (*molt*) their exoskeletons at intervals. Consequently in some specimens which were preparing to molt when killed, a new soft exoskeleton may be found in process of formation between the epidermis and the old exoskeleton.

b) *Muscular system:* The lobster or crayfish has well-developed muscles. These will be found attached to the exoskeleton as the latter is being removed. In the anterior region of the body under the carapace is the large thin-walled *stomach* from the anterior and posterior ends of which *gastric* muscles extend to the carapace. At each side of the posterior end of the stomach is a heavy mass of muscle, the *mandibular* pair of muscles, which move the mandibles. In removing the exoskeleton of the abdomen note the longitudinal muscles attached to its inner surface in each segment and trace them forward to their origins on the sides of the thorax. They are the *extensors* of the abdomen which serve to straighten the abdomen. After the removal of the whole dorsal exoskeleton of the abdomen and the muscles attached to it, large masses of ventral abdominal muscles will be noticed. These are the *flexors* of the abdomen, which bend the abdomen ventrally. Why are they more powerful than the extensors? Remove the left thoracic wall without injuring any of the internal parts and trace the flexor muscles forward to their origins from the lateral and ventral thoracic walls. Are the abdominal muscles segmentally arranged?

Each appendage is supplied with muscles. To illustrate their arrangement examine the muscles of the chela. Remove the exoskeleton from one surface of the chela and note in the chela two muscles, a very large *flexor* and a much smaller *extensor*. Find out by pulling on these muscles their attachments and action on the movable part of the chela. Each joint of each appendage is similarly provided with a flexor and extensor muscle for moving the next joint distal to it.

We observe that the muscular system of the animal is highly developed as compared with that of other invertebrates studied. In the lower forms the muscular system consists mostly of simple tubes or layers of muscle fibers, producing merely extension, contraction, or bending of the body. But in the lobster, as in the frog, there are present definite muscles having specific action on various parts of the body, permitting much greater variety and exactness of movement.

Place a small bit of muscle on a slide in a drop of water, tease into fibers, cover, and examine. Are the fibers cross-striated as in the frog?

The *body wall* of the animal may be considered to be composed of the epidermis with its exoskeleton and the layer of muscles. However, owing to the absence of a coe-

lome (see below) the body wall is not very sharply marked off. A peritoneum is missing.

c) *The circulatory system:* Your specimen should now have the dorsal and one lateral wall cleaned away so as to expose the viscera. The conspicuous organs are the stomach anteriorly, the *heart* posterior to this, usually injected with yellow material and with yellow vessels springing from it, and a large white organ, the *digestive gland,* occupying the sides of the space within the thorax and extending back into the abdomen.

The space around the heart and between the viscera is not a coelome, as the student might expect, but is an enormous blood space, or *blood sinus,* filled in life by blood. The coelome has been greatly reduced, in fact, is practically absent through this great development of blood sinuses. The studies which we have made on the coelomes of other animals should convince the student that this space in the lobster cannot be a coelome because mesenteries are completely lacking. Note that none of the organs are supported by mesenteries and that there is no peritoneal lining.

The heart lies free in a large sinus, the *pericardial sinus.* The following arteries arise from it. From the anterior end of the heart in the median line springs the single *ophthalmic artery.* Trace it forward along the dorsal surface of the stomach and note its branches to the eyes. On each side of the ophthalmic artery arises an *antennary* artery which curves downward over the digestive gland. Trace it and note its branches to the mandibular muscle, the stomach, the antennae, and the antennules. Directly below the origin of the antennary arteries and from the ventral surface of the heart the paired *hepatic* arteries extend down into the substance of the digestive gland. From the posterior end of the heart a large vessel, the *dorsal abdominal* artery, extends backward the whole length of the abdomen, forking in the sixth abdominal segment. It gives branches to the intestine which is immediately beneath it and to the extensor muscles of the abdomen which have been removed. Are its branches segmentally arranged? From the posterior end of the heart at the same point as the origin of the dorsal abdominal artery, another large artery, the *sternal* artery, arises and proceeds directly ventrally. Push the left digestive gland carefully aside to see it. Its further course will be traced later.

Make an outline of the lobster from the side as large as your drawing page and in this put the heart and its arteries. Other systems will be added to this later.

Remove the heart from the body, wash it in water, and note its peculiar angled form. It is generally somewhat distorted by the injection. Find the three pairs of openings, or *ostia,* in the wall of the heart. One pair is on the dorsal surface, one pair on the ventral surface, and one pair is lateral just under the lateral margins.

The circulatory system of the lobster and other arthropods is an *open* system, that is, the only definite vessels are the arteries, and the circulation is completed through open spaces, or sinuses. In the lobster the blood passes from the arteries into these sinuses and finally collects into one large sinus, the *sternal* sinus, in the median ventral line. This will be seen later. It then passes into the gills by the *afferent* vessels, out by the *efferent* vessels, already seen in the cross-section of a gill, and back to the peri-

cardial sinus by definite channels. When the heart expands the ostia open and blood is sucked into the heart from the pericardial sinus. The blood is colorless.

The ophthalmic artery, heart, and dorsal abdominal artery together correspond to the dorsal vessel of the earthworm. In the earthworm, it will be recalled, the entire dorsal vessel is contractile, but in the lobster the power of contractility has become limited to one region, which is enlarged and now designated as a heart. There is also in the lobster a vessel to be seen later which corresponds to the ventral vessel of the earthworm. The student should particularly notice that the segmental vessels, connecting these two in each segment of the earthworm, are for the most part lacking in the lobster.

d) *The reproductive system.* Lying under the heart will be found a pair of slender gonads, white in the male, pinkish in the female. They extend forward almost to the anterior end of the stomach and posteriorly beyond the termination of the digestive gland, in the lobster. (In the crayfish they are shorter and broader, and the posterior part is single.) The two gonads have a slender connection just in front of the heart. In the female an *oviduct* will be found arising from each ovary in the region of the heart. Trace it downward over the surface of the digestive gland into the third leg. In the male the *vas deferens* arises from the testis in the same region and extends backward to the fifth leg. Soon after leaving the testis the vas deferens presents a bend, then widens and proceeds straight to the external opening. (The vas deferens of the crayfish is greatly coiled.) **Draw in the reproductive system in your lateral view of the lobster.**

e) *The digestive system.* The conspicuous parts of the digestive system are the large thin-walled stomach and the voluminous digestive gland. The latter, a paired white organ filling the greater part of the cavity of the cephalothorax, is often called a "liver," but as it really combines the functions of both a liver and a pancreas it would be more appropriately designated the *hepato-pancreas*. Observe the lobes and small tubules of which it is composed. Note shape, size, and extent of the digestive gland and enter it on your drawing with very light lines. Then remove the left one completely, noting as you do so the place where it is attached to the posterior end of the stomach by an *hepatic duct*.

The removal of the digestive gland exposes the stomach more fully. Observe by pushing the stomach over to the right the short *esophagus* connecting it with the mouth. The stomach is divided by a constriction into a large anterior *cardiac* portion, whose wall contains hard *ossicles,* and a much smaller posterior *pyloric* portion. The hepatic ducts open into the pyloric portion just above the rounded processes which project downward from its sides. Fastened to each side of the cardiac portion of the stomach will often be found a large mass of crystals, calcareous in composition, called a *gastrolith,* perhaps stored for later use in making new shells. From the end of the pyloric chamber find the slender *intestine* and trace it to the anus. It makes a deep ventral bend just behind the stomach and then ascends to a position directly beneath the dorsal abdominal artery. In the sixth abdominal segment it gives off a blind dorsal sac, or *caecum;* from this point to the anus the intestine is called *rectum.* **Draw in these parts of the digestive system on your outline of the lobster.**

Remove the stomach, leaving the esophagus in place. Cut it open, wash it out, and examine the interior. Note the hard pieces, or *ossicles,* in the walls of the cardiac chamber, and the paired *lateral* and single *median teeth* and the projecting processes covered with fine silky hairs in the posterior division of the chamber, blocking the passage into the intestine. The teeth and ossicles are a grinding apparatus, called the *gastric mill,* operated by the *gastric muscles* attached to the anterior and posterior ends of the stomach; the hairy processes, a straining apparatus, preventing the coarser particles from passing into the intestine. **Make a drawing** showing the interior of the stomach.

f) *The excretory system:* In the anterior end of the body cavity, internal to the base of the antenna, locate a circular greenish mass, liberally supplied by branches of the antennary artery. This is the excretory organ, commonly called "green gland." It is in reality a modified nephridium, which has lost its funnel-shaped opening into the coelome (since there is no coelome). The renal opening in the basal joint of the antenna has already been observed. The student should note that while in the annelids there is a pair of such nephridia in practically every segment, in the lobster they are present in one segment only, the antennary segment. This is another example of the loss of segmental structures, which characterizes the arthropods as compared with the annelids. **Draw** in the green gland on your side view.

g) *The nervous system:* Remove all soft parts from the interior, leaving the esophagus and sternal artery in place. First begin in the abdomen and carefully remove all of the ventral abdominal muscles, noting their segmental arrangement and the complex manner in which they loop over one another. In the median ventral line of the abdomen, next to the inner surface of the shell, is a white cord, the *ventral nerve cord.* In the thoracic region this will be found to disappear into a cavity which is roofed over by hard plates. Clean out the muscles and other soft parts in the thorax until you have exposed these plates. They form the so-called internal or *endophragmal* skeleton; this is not a true endoskeleton but consists of ingrowths from the exoskeleton.

The cavity beneath the endophragmal skeleton is the *sternal sinus,* which was mentioned in connection with the circulatory system as the large sinus in which all of the venous blood collects before passing to the gills. In this sinus are located the thoracic part of the nerve cord and the greater part of the sternal artery. Remove the endophragmal skeleton by cutting along each side and taking out the middle piece. Trace the nerve cord forward into the sternal sinus to the esophagus. Note that the nerve cord forks at the esophagus into the *circum-oesophageal commissures.* These encircle the esophagus and unite again into a bilobed mass, situated internal to the region between the two eyes, and somewhat concealed by strands of tissue which should be carefully picked away.

The bilobed mass in question is the *brain,* better designated as the *supra-esophageal ganglia.* It sends nerves to the eyes (where they expand into the *optic ganglia,* situated in the eye stalks), the antennules, and the antennae. The brain, therefore, represents three pairs of ganglia fused together. From the brain the circum-esophageal commissures encircle the esophagus as noted above and unite behind the esophagus into a large mass, the *sub-esophageal ganglion.* Note branches arising from the com-

misures and sub-esophageal ganglion. Trace the nerve cord posteriorly along the floor of the sternal sinus and note its enlargement into a ganglion at intervals. Behind the sub-esophageal ganglion there are three ganglia; the nerve cord then forks to allow the sternal artery to pass through; behind the sternal artery are two more thoracic ganglia. Since in a segmented animal there is a ganglion (really a pair) to each segment, how many ganglia should be present in the cephalothorax of the lobster? Since the brain supplies the eyes, antennules, and antennae, and since each thoracic ganglion supplies one pair of thoracic appendages, how many appendages and which ones must the sub-esophageal ganglion supply and of how many fused ganglia does it then consist?

Trace the nerve cord into the abdomen. Is there a ganglion for each abdominal segment? Does the telson have a ganglion? Note branches of the abdominal nerve cord.

Study the sternal artery. As noted, it passes through the nerve cord, between the fourth and fifth thoracic ganglia and promptly divides into an anterior branch, the *ventral thoracic* artery, and a posterior branch, the *ventral abdominal* artery. These correspond to the ventral vessel of the earthworm. Note branches from them into the appendages, etc.

Put the nerve cord and its ganglia and the course of the sternal artery into your drawing. The drawing is now complete.

The nervous system of the lobster is very much like that of the earthworm except that as in the case of the other systems of the lobster it shows a partial loss of the segmental arrangement through fusion. The nervous system of both earthworm and lobster is based upon the same plan as that of planarians, consisting fundamentally of two ventral ganglionated cords arising from a dorsal brain. This type of nervous system is common to all of the invertebrates (except those having radial symmetry) and is called the *ladder type*. The ventral nerve cords, originally separated rather widely, come together in the segmented invertebrates to produce an (apparently) single cord.

3. Behavior of the crayfish.—Crayfish are easily obtained from ponds and streams. Build up a sandbank at one end of an aquarium, pile rocks here and there on the bottom in such a way as to leave spaces under the rocks, and place several living crayfish in the aquarium.

a) *Locomotion:* Observe method of walking. Which appendages are used? How are the others held? Can the animal walk sidewise or backward? Cause the animal to swim. What is the chief swimming movement? Explain action of muscles of the abdomen in swimming. Are the pleopods used in swimming? Are they kept in motion when not swimming? Explain purpose.

b) *Respiratory movements:* Observe in a quiet specimen the continual rhythmic movement of certain appendages. Determine which appendages are concerned. These movements are respiratory in nature. Explain how. From your study of the anatomy of the animal can you mention any other movements of the animal that would assist respiratory exchange? With a dropper place a few drops of carbon or carmine suspension near the posterior edge of the branchial region of the carapace. By the movements of the granules determine if there are any respiratory currents present and in which direction they go.

c) *Response to touch:* With a glass rod drawn out into a blunt point touch various parts of the animal and note responses. Touch various appendages. Is the response different according to the part touched? Are some regions or appendages more sensitive than others?

d) *Attitude of defense:* Make a strong threatening movement in front of the animal and note attitude assumed by the animal.

e) *Thigmotaxis:* What use do the animals make of the sandbank? of the spaces under the stones? Note attitude assumed.

f) *Feeding:* Drop a piece of fresh raw meat four or five inches from a crayfish. How long before the animal perceives the presence of food? Note its behavior and observe method by which it finds the food. Do you think it sees, feels, or smells the food? Experiments have shown that the antennules have the greatest sensitivity to chemical substances; the antennae, the small pincers of the pereiopods, and the appendages around the mouth are also sensitive but less so. Observe process of chewing the food and use of the various mouth appendages in the process. In nature the crayfish catches and eats small animals such as tadpoles, snails, insect larvae, and also feeds on dead animals and vegetable matter. It is said to feed chiefly at night.

g) *Reproduction:* Females carrying eggs on the swimmerets are commonly found in the spring. Observe movements of the swimmerets. What is their function? Observe to what and how the eggs are attached. Hatching occurs in six to eight weeks from the laying of the eggs. At hatching the larva is attached to the mother for some time by a thread. Examine newly hatched larvae and observe differences from adult form. Note later changes of form subsequent to molting. Observe behavior of the young with reference to the mother.

4. General considerations on the lobster.—The chief principle which we wish to bring out through the study of the anatomy of the lobster has already been emphasized throughout the laboratory instructions. It is that the lobster, although a segmented animal like the annelids, exhibits a marked tendency to an obliteration of the segmentation, through fusion and loss of segments and of segmented structures. This tendency is still further in evidence in the vertebrates where dissection alone reveals that the frog is segmented, and then in only a few systems. What systems of the lobster are segmented? Which is the most completely segmented? In what part of the body is the segmentation most complete? Where is it most obscure? Compare with the frog and determine whether the systems and the part of the body which exhibit the greatest degree of segmentation are the same in the two animals. Is it the same system in the two which retains the most primitive segmentation? What is the significance of this fact? Does the lobster have all the systems which are present in the frog? Which does it possess which the earthworm lacked? Are the systems which the earthworm and the lobster have in common better developed and more specialized in the latter? Which one differs the least from that of the earthworm? Do the systems of the lobster compare favorably with those of the frog as to specialization for particular functions? What system of the lobster has made the least progress? Do both the lobster and the frog seem to have attained a fairly high degree of adjustment (adaptation) to the conditions under which each lives?

B. ANATOMY OF THE GRASSHOPPER

The insects constitute the class Hexapoda of the phylum Arthropoda. They are distinguished by the presence of but three pairs of walking legs, one pair of antennae, and a respiratory system of tracheal tubes. The grasshopper is commonly studied as a representative of the insects. The following description applies particularly to the large southern grasshoppers, commonly used in biological courses because of their large size.

1. External anatomy.—Obtain a preserved specimen. Compare with the earthworm and especially with the lobster or crayfish. Is the animal bilaterally symmetrical? Is it segmented throughout? In what part of the body is the segmentation most apparent? least apparent? Does the grasshopper have *jointed appendages* like the lobster or crayfish? Do they occur on every segment? What part of the body appears to lack them? Does the animal have a well-developed *head?* Compare with other animals studied as to the differentiation of a head. Is a definite color pattern present?

The body is covered by an exoskeleton composed of *chitin*, secreted by the underlying epidermis. It consists of hard regions, or *sclerites*, joined together by thin membranes, the *arthropodial membranes*, or *conjunctivae*. When the conjunctivae are reduced to mere lines, these lines are known as *sutures*.

The body is divided into three definite regions—*head*, *thorax*, and *abdomen*. Are these three regions more distinct from each other than in the lobster? Each region consists of a definite number of *segments*. Each segment as in the lobster is typically composed of a dorsal part, the *tergum*, a lateral part, the *pleuron*, and a ventral part, the *sternum*. Each of these may consist of more than one sclerite.

a) The head and its appendages: Is the head readily movable upon the body? The head appears to be unsegmented. The larger part of it is inclosed in one continuous hard case, in which may be distinguished a top (*vertex*), sides (*genae*), and a front (*frons*). There are no distinct boundaries between these regions in the grasshopper. Looking at the head from in front, the lower limit of the frons is seen to be marked by a distinct transverse suture. Below this suture is a rectangular sclerite, the *clypeus*, and below this and attached to it another sclerite, the bilobed *upper lip* or *labrum*. Observe that the labrum is movable; it is not, however, an appendage, but one of the sclerites of the head.

The head bears *eyes*, *antennae*, and three pairs of *mouth parts*. There are two kinds of eyes, *compound* and *simple*. The compound eyes are the large eyes situated in the upper parts of the genae. Examine one with a hand lens and note the numerous minute hexagonal areas or facets into which the surface is divided. Each facet is the outer end of an *ommatidium*. For the structure of ommatidia, texts should be consulted. There are three simple eyes or *ocelli*, small brown disks situated as follows: one anterior to the dorsal portion of each compound eye on the ridge which separates frons and gena, and the third in the depression between the two ridges in the median line of the frons. **Draw a front view** of the head.

The antennae are the first pair of head appendages. They are jointed filamentous structures, springing from depressions in the upper part of the frons. The antennae are very important olfactory and tactile appendages. Remove an antenna and examine with low power, noting sensory hairs on it.

With a forceps remove the clypeus and labrum in one piece and examine the under surface. This forms the roof of the mouth and bears a central elevation, the *epipharynx*, probably of sensory function.

The removal of the labrum exposes the three pairs of mouth parts. These are named the *mandibles*, *first maxillae*, and *second maxillae*, and constitute the second, third, and fourth pairs of head appendages, respectively. The mandibles are the hard brown structures with toothed inner edges. Remove one and draw under a hand lens. The first maxillae are beneath the mandibles and bear projecting processes, the *maxillary palps*. Remove a maxilla complete and study it with a hand lens. It consists of a basal portion, composed of two segments, a lower *cardo* and an upper *stipes*, from which springs three processes. The inner process is a curved, toothed blade, the *lacinia;* the middle one an oval two-jointed plate, the *galea;* the outer, a slender jointed structure, the *maxillary palp*. Draw the maxilla. The last pair of mouth parts consist of the two second maxillae partially fused in the median line to form a structure called the *labium* or *lower lip*, which forms the posterior surface of the mouth cavity. Attached to the labium and projecting into the mouth cavity is an elevation, the *hypopharynx*, which serves as an organ of taste. Remove the labium complete and identify its parts under a hand lens. It consists of a basal crescentic joint, the *submentum*, followed by a small narrow neck, the *mentum*, followed by two large flaps, the *ligulae;* from the side of each ligula springs a jointed process, the *labial palp*. Draw the labium.

Since the head bears four pairs of appendages it must consist of at least four segments. Investigation of insect embryos has shown that there is another segment in front of the one bearing the antennae, to which the compound eyes belong, and still another between the antennary and mandibular segments corresponding to that one in the lobster which bears the antennae. This segment and appendages are lost in early stages of development, the antennae of insects corresponding to the antennules of crustaceans. There are thus at least six segments in the insect head. Some authorities believe there is yet another segment present between the mandibular and maxillary segments, making a total of seven.

b) *The thorax and its appendages:* The thorax is composed of three stout segments, called the *prothorax*, *mesothorax*, and *metathorax*, respectively, beginning at the anterior end. The tergum of the thoracic segments is commonly called *notum* in insects. The *pronotum* (i.e., notum of the prothorax) is greatly enlarged and extends back like a shield over the mesonotum and metanotum. The sternum of the prothorax has a sharp posteriorly directed spine. The pleuron of the prothorax is rudimentary. The prothorax bears the first pair of legs. Is the prothorax independently movable?

Cut away the backward extension of the pronotum. In the middle of the side in the suture between the prothorax and mesothorax, find an oval opening. This is a *spiracle* or *stigma*, one of the openings into the respiratory system. In the mesothorax, identify the dorsal tergum or notum, the lateral pleuron, and the broad ventral sternum. Both notum and pleuron are composed of several sclerites. The mesothorax bears dorsal outgrowths, the first pair of wings, and a ventral pair of appendages, the second pair of legs. Cut off the wings.

The metathorax is similar in form and parts to the mesothorax and bears the sec-

ond pair of wings and the third pair of legs. Cut off the wings. Locate another spiracle between the ventral parts of the pleura of the mesothorax and metathorax.

Examine the wings. They are rather short in the large southern grasshoppers, which are consequently known as *lubber* grasshoppers because they cannot fly. Compare the two pairs of wings as to form, size, color, thickness. What do you consider to be the functions of each pair? Note the so-called *veins* in the wings. Each is really an *air tube* or *tracheal tube* surrounded by a space, which is a *blood sinus*. The air tube and blood sinus function only during the development of the wing, the adult wing being dead. Examine a vein in a piece of the second pair of wings under the microscope and note the ringed air tube and the blood sinus composing the vein.

Remove a leg from the body noting the depression in the body where it fits, the conjunctiva which attaches it to the body, and the muscles at its base. The leg is composed of five segments. The rounded segment which adjoins the body is the *coxa*. It is succeeded by a very small joint, the *trochanter*. The next segment is the long powerful *femur*. Beyond this comes the more slender spiny *tibia*. The terminal part of the leg is the *tarsus*, consisting of three joints with five ventral pads, the *pulvilli*, and a terminal pair of hooks, the *tarsal claws*. Compare the three pairs of legs of the grasshopper with each other. Do all have the same parts? Can you associate differences in relative proportions of the five joints with differences in function? Suggest uses for the pulvilli and tarsal claws.

c) *The abdomen and its appendages:* The abdomen of insects consists of a definite number of segments, eleven according to most authorities, twelve in the opinion of some. The complete number of segments is, however, rarely apparent externally owing to the fact that the terminal segments are telescoped into the interior forming parts of the genital apparatus. Each abdominal segment consists of a tergum, shaped like an inverted **U** and forming the dorsal and part of the lateral wall of the segment, and a sternum, the convex ventral plate. The pleuron is lacking. An infolded conjunctiva joins tergum and sternum. Just above this line of junction on the lower border of the tergum will be found a spiracle. How many pairs of spiracles are present on the abdomen?

Study the first abdominal segment. Its tergum and sternum are separated from each other by a depression for the insertion of the metathoracic legs. The sternum is fused to the sternum of the metathorax, a crescentic suture, however, marking the boundaries between them. The tergum bears upon each side a circular opening with a chitinous rim across which is stretched a thin membrane. This is the organ of hearing or *chordotonal organ*, commonly called an "ear." On the anterior border of the ear is the first abdominal spiracle.

The next seven abdominal segments are all alike and present no new features. The remainder of the abdomen is different in form and structure in the two sexes. In both sexes the ninth and tenth terga are narrow and more or less fused. From the tenth a broad triangular plate, the *supra-anal* plate, extends posteriorly, forming the roof of the anal opening. This plate is divided (in some grasshoppers) into two parts by a transverse groove; by some authorities its anterior half is considered as the eleventh tergum, its posterior half as the twelfth tergum; while others regard the entire plate as

representing the eleventh tergum. On each side below the supra-anal plate is another triangular plate, the *podical plates* or *paraprocts,* parts of the eleventh segment. A process called a *cercus* or *cercopod* projects posteriorly on each side from the membrane between the tenth tergum and the paraproct. These cerci are different in form in males and females and in males are different in shape in each species of grasshopper. They are the appendages of the eleventh abdominal segment. The *anal opening* is between the two paraprocts.

In the male, the ninth sternum is very large and bears at its posterior end a hood-shaped piece, the *subgenital plate,* which terminates dorsally in two short projections. The terminal part of the male genital apparatus is concealed under the subgenital plate (in some specimens it may be protruded) which forms a hood over it. If necessary grasp the subgenital plate and pull it downward. A fleshy mass, the end of the male reproductive system is revealed. From it projects a hard spinelike process, the *penis* or male mating organ.

In the female there is a broad eighth sternum from the middle of whose posterior edge projects a short pointed process, the *egg guide.* The abdomen terminates in two pairs, *dorsal* and *ventral,* of hard pointed projections called *valves.* On spreading these two pairs of valves apart a much smaller third or *inner* pair of valves is seen between their attached ends. These three pairs of valves constitute the *ovipositor* or egg-depositing apparatus of the insect. They are the appendages of the eighth, ninth, and tenth abdominal segments. The grasshopper lays its eggs in holes in the ground. Does this suggest the function of the dorsal and ventral valves?

2. **Internal anatomy.**—The dissection of preserved specimens is rather unsatisfactory. For this reason the student may have some difficulty in locating all of the parts described and should not waste too much time in searching for them.

a) *Body wall and internal cavity:* Cut out the dorsal body wall in one strip by making a cut on each side, beginning in front of the supra-anal plate and extending forward to the compound eyes. Carefully remove this strip being sure not to take any of the internal organs with it. By examining this strip determine that the body wall consists of the chitinous exoskeleton (with the underlying epidermis invisible to the naked eye) and a layer of muscles. Keep the strip for future study. The removal of the strip reveals a cavity in the body containing viscera. As in the case of the lobster this cavity is not a coelome but an enormous *blood sinus;* this is evidenced by a complete lack of mesenteries supporting the viscera and by the absence of a lining peritoneum. A yellowish material, the *fat body,* representing stored food, may be noticed among the viscera and clinging to the body wall.

b) *Muscular system:* The muscular system is complicated. We shall here note merely that there are numerous muscles on the inner surface of the body wall and slender bandlike muscles for moving the appendages. Each joint of the appendages contains muscles similar to those seen in the lobster.

c) *Respiratory system:* There is perhaps no other structure to be found in the animal kingdom which so excites the admiration of the zoölogist as the respiratory system of insects. Unfortunately it is impossible to study it satisfactorily in preserved material and freshly killed specimens should be obtained if possible. This respiratory

system found only in insects and a few related arthropods consists of a series of air tubes, symmetrically and segmentally arranged, called *tracheal tubes* or *tracheae*. These open to the exterior through the spiracles or stigmata already noted. How many pairs of spiracles are present in the grasshopper? To see the tracheal tubes, pull one side of the body wall gently outward, press the viscera back in the opposite direction, and look on the inside of the body wall internal to the location of the spiracles. A white tube should be seen leading inward from each spiracle. With a little practice one will soon be able to identify similar tubes throughout the body. Note also particularly in the thorax the large white *air sacs* which are connected with the tracheae and serve to increase the capacity of the tracheal system. For details of the tracheal system of the grasshopper study figures in texts. Recall also the tracheal system seen in the fly larvae (chapter ii, F).

Remove a piece of a tracheal tube, mount in a drop of water on a slide, and examine with low power. Note the spiral thread on the inside of the tracheal tube. What is its function? **Draw.**

The tracheal system is full of air which by means of the ramifications of the tracheal tubes reaches the cells of the body directly. This system appears to be more efficient than the usual system found in other animals, in which the air is carried in the blood. The air in the tracheal tubes is changed by means of respiratory movements comparable to human inspiration and expiration. These should be observed if possible on living grasshoppers. According to recent investigations air enters through the thoracic and first two pairs of abdominal spiracles and exits through the other abdominal spiracles. The spiracles can be opened and closed by means of valves.

d) *Circulatory system:* Associated with the development of a remarkably efficient respiratory system there is a correspondingly poor differentiation of the circulatory system. There is a long segmented *heart* situated in the median dorsal line of the abdomen. Look for it on the under surface of the dorsal strip which you removed from the animal. It is a long tubelike structure with several dilatations. It is often indistinguishable in specimens that have been preserved a long time. Fan-shaped muscles, the *alary muscles,* are attached to each dilatation and are believed to help the heart expand. As in the lobster the wall of the heart is pierced by ostia, impractical to see, through which blood is sucked into the heart from the surrounding blood sinus at each expansion.

The posterior end of the heart is closed. Its anterior end continues forward into the head as a slender tube sometimes called an *aorta*. This opens into body spaces through which the blood flows finally collecting in the sinus around the heart.

e) *Reproductive system:* This system will be considered next since it is the most dorsally situated of the systems. In the male the single *testis* forms a white or yellow mass in the posterior part of the abdomen immediately under the dorsal body wall. From its posterior end on each side arises a slender duct, the *vas deferens,* which passes posteriorly and ventrally. Internal to the eighth and ninth sterna each vas deferens receives a large mass of coiled tubules which are said to consist in part of *seminal vesicles* or storehouses of sperm and in part of *accessory glands* of uncertain function. To see these structures pull the lateral body wall outward and push the internal organs to

the opposite side. After receiving these tubules the vasa deferentia of the two sides unite in the mid-ventral line in the ninth segment to form a rather wide single duct, the *ejaculatory duct,* which passes into the muscular mass surrounding the penis. The ninth sternum and subgenital plate may be cut away to see these parts. Remove the muscles from the penis and note with a hand lens the four hard chitinous *styles* of which it is composed.

In the female the *ovaries* form two large masses filling the dorsal part of the abdomen. Free the two ovaries from the fat body and other tissues and note that each consists of a number of parallel *ovarian tubules,* more or less vertical in position, their ventral ends attached to the *oviduct* which runs horizontally. In each ovarian tubule is a row of *eggs,* of which the largest one, plainly seen as an elongated brown body, fills up the greater part of the tubule. The two oviducts pass posteriorly and ventrally and unite in the median line under the intestine to a common duct, the *vagina.* Follow one oviduct by pulling the body wall widely away from the internal organs which should be pressed to the other side. Note the union of the two oviducts to form the vagina which lies internal to the eighth sternum. Follow the vagina to its external opening on a little cushion at the base of the egg guide. Note just dorsal to the vagina another tube, the *copulatory pouch,* which opens to the exterior between the bases of the two ventral valves of the ovipositor. The copulatory pouch leads inward into a rounded sac, the *spermatheca* or *sperm receptacle,* in which the sperm received at copulation (sexual union) are stored. Attached to the spermatheca are coiled tubules, the *spermathecal glands,* of uncertain function. In laying eggs the grasshopper digs a hole in the ground with the dorsal and ventral styles of the ovipositor. As the eggs pass out they receive sperm from the opening of the copulatory pouch. The egg guide and the inner valves of the ovipositor direct the eggs in their passage into the hole, arranging them in packets.

Draw from the side the parts of the reproductive system which you have been able to find.

f) Digestive system: The larger part of the internal cavity is filled with the digestive tract. Remove the reproductive system. Clean away the fat body and cut away one lateral side of the abdomen, thorax, and head. Identify the short *esophagus* extending inward from the mouth and opening into a large *crop* which fills most of the cavity of the thorax. Behind the crop is the elongated *stomach* or *ventriculus,* partially concealed by six thin-walled *gastric caeca.* Each gastric caecum is attached at its middle to the wall of the stomach, one of its pointed ends projecting forward, the other backward. It is probable that these caeca secrete a digestive fluid containing enzymes into the stomach. The anterior part of the stomach covered by the anterior halves of the caeca is distinguished as the *proventriculus* or *gizzard* but is not well differentiated in the grasshopper. The posterior end of the stomach is marked by the presence of a tangle of threads, the *Malpighian tubules.* Beyond this region is the *intestine,* at first wide, then presenting a narrow portion, the *colon,* then widening into the *rectum* which extends to the anus. The rectum frequently contains a cylindrical pellet of feces. Its surface is marked off by longitudinal muscle bands into six expanded areas, known as the *rectal glands,* of uncertain function. The *anus* is situated under the supra-anal

plate. Look in the thorax at each side of the crop for a *salivary gland,* a cluster of small round glands attached to a duct which opens into the mouth.

Draw an outline of the animal from the side and put in the parts of the digestive tract. Other systems besides the reproductive system which the student has seen well enough may also be entered on this drawing.

g) *Excretory system:* The threadlike Malpighian tubules arising at the junction of stomach and intestine are the excretory organs. They are outgrowths of the digestive tract and, therefore, do not correspond to the excretory organs of any other animals that we have studied. The insects have lost the nephridia.

h) *Nervous system:* Cut through the esophagus leaving it in place and remove the digestive tract. Look in the median ventral line of the abdomen against the ventral wall for the *ventral nerve cord.* How many ganglia are present in the abdomen? Trace the nerve cord forward into the thorax. Note here, as in the lobster, a so-called endoskeleton, really an ingrowth from the exoskeleton, extending like beams across the floor of the thorax. Remove this and muscles, etc., so as to expose the thoracic part of the nerve cord. Find the three large *thoracic ganglia,* one for each segment of the thorax. Anterior to the prothoracic ganglion is the *sub-esophageal ganglion.* From this the two *circum-esophageal commissures* pass forward, one on each side of the esophagus, and unite at the *brain,* or *supra-esophageal ganglion,* located on the inner surface of the head between the two compound eyes. Does the nervous system of the grasshopper resemble that of the lobster? earthworm?

Draw in the nervous system on your lateral view of the grasshopper.

i) *Sensory system:* The chief sense organs were already noted in the study of the external anatomy. For further information on the numerous and varied sense organs of insects, texts should be consulted.

3. General considerations on the grasshopper.—What are the chief differences between the external anatomy of the grasshopper and the lobster? What internal systems of the two animals show the most differences? resemblances? What systems of the grasshopper are segmented? What particularly highly effective and highly specialized systems does the grasshopper possess? From your study of the anatomy of the grasshopper can you give reasons why the insects are the most successful and dominant animals on the earth, excepting man?

C. GENERAL SURVEY OF ARTHROPODS

Examine the specimens and compare them with each other and the lobster and grasshopper as to degree of segmentation, specialization and number of appendages, general forms, symmetry, and divisions of the body. The common groups of arthropods are:

1. Crustacea, forms with two pairs of antennae, many pairs of appendages, hard crustaceous exoskeleton, and gills as respiratory organs. Examine the following representatives (not arranged by classification).

a) *Entomostraca,* small to microscopic Crustacea, common in fresh water, often inclosed in a bivalve carapace. Preserved forms are of little value; living forms if available will be demonstrated.

b) *Barnacles,* sessile forms, inclosed in calcareous plates. Marine forms, covering the rocks along the seashores.

c) *Shrimps,* marine animals similar to crayfishes, but with more slender, delicate build.

d) *Crabs,* marine animals like the lobster, but with a much broadened cephalothorax, and reduced abdomen folded under the cephalothorax.

e) *Amphipods,* small fresh-water animals, something like miniature crayfishes, but strongly compressed from side to side.

f) *Sow bugs* or *pill bugs,* small forms, greatly compressed dorso-ventrally, common in damp places, greenhouses, etc., also in water; curling into balls when disturbed.

2. **Arachnids,** forms without antennae, and four pairs of walking legs.

a) *Spiders,* require no description.

b) *Scorpions,* with segmented abdomens narrowing to a long tail bearing a terminal sting.

c) *Mites,* small, flattened forms, having lost all trace of segmentation, generally external parasites.

3. **The king crab, or horseshoe crab,** survivor of an ancient, mostly extinct, group, with a large, rounded cephalothorax; abdomen terminated by a long sharp spine.

4. **Insects,** with one pair of antennae, three pairs of walking legs, breathing by means of tracheae.

5. **Millipedes and centipedes,** elongated forms consisting of many segments. Each segment has one pair of legs in the centipedes, and two pairs in the millipedes.

XV. PHYLUM CHORDATA: SPECIAL ANATOMY OF THE FROG

The phylum Chordata is placed at the top of the animal kingdom because of the great complexity of structure and remarkable development of the nervous system of its members. This phylum includes all of the animals known as *vertebrates* together with a few other small groups which have been found to be related to vertebrates. A vertebrate is an animal which possesses an internal skeleton of cartilage or bone, composed of several parts, one of the most characteristic of which is the vertebral column. This is an elongated skeletal structure, situated in the sagittal plane, and composed of a succession of similar pieces, known as vertebrae. In the embryos of the vertebrates the vertebral column is represented by an elastic rod, the *notochord,* around which the vertebrae later form. All animals which possess neither a notochord nor a vertebral column are known as *invertebrates.* All of the animals hitherto studied in this course except the frog are invertebrates.

At the beginning of this book the anatomy of the frog was surveyed in a general way in order to furnish the student with a conception of the construction of an animal and the functions of its parts. The anatomy of the frog will now be taken up in some detail as a representative of the phylum Chordata.

A. THE DIGESTIVE SYSTEM

The digestive system begins at the mouth and ends at the anus. We have already seen most of the parts of this system. Use the frogs left from the work of chapters i and ii.

1. **Buccal cavity and pharynx.**—The contents of these cavities were sufficiently described in chapter i. We shall here note particularly the characters of the *teeth.* The teeth are structures characteristic of vertebrates. They are composed chiefly of bone, the same material of which the skeleton is constituted. Examine with a hand lens the teeth on a dried skull of the frog and note the following points. The teeth of the frog are not set into *sockets,* that is, cavities in the jaw, as are our own teeth but are fused by their bases and sides to the margins of the jaw in such a way that their ends project freely beyond the edge of the bone. Each tooth is a hollow cone, consisting of two parts separated by an indistinct groove: an outer part, the *crown,* coated externally with a shiny material, the *enamel;* and a basal part, the *root,* fastened to the jaw. The teeth of the frog are not used in chewing food but for grasping prey. They are replaced an indefinite number of times when lost and sets of new teeth can usually be seen at the bases of the old ones.

2. **Esophagus.**—The cavity of the pharynx continues into a tube, the *esophagus.* On a preserved frog, cut through the tissues bounding the anterior wall of the coelome to the left of the left lung and turn the left lung and heart to the right. This exposes the esophagus. It is a stout tube extending from the end of the pharynx to the stomach. A slight constriction usually marks the beginning of the stomach.

3. Stomach.—The end of the stomach continuous with the esophagus is the *cardiac* end; the opposite end is the *pyloric* end and is marked by a constriction, the *pylorus*. The stomach is fastened to the dorsal wall of the pleuro-peritoneal cavity by a mesentery, the *mesogaster*. Slit open the stomach by a longitudinal slit and note the longitudinal ridges or *rugae* in its lining.

4. Small intestine.—The small intestine is the slender tube extending away from the pyloric end of the stomach. Immediately beyond the stomach it makes a bend anteriorly toward the liver. This part of the small intestine is known as the *duodenum*. A mesentery, the *gastro-hepato-dudodenal ligament* extends between duodenum, stomach, and liver. Beyond the duodenum the remainder of the small intestine, known as the *ileum*, is more or less coiled. The small intestine is attached to the mid-dorsal wall of the coelome by a mesentery continuous with the mesogaster and known simply as the *mesentery* proper.

5. Liver.—The liver is a large gland attached to the duodenum and stomach by the gastro-hepato-duodenal ligament mentioned above, to the dorsal wall of the coelome by a mesentery, the *mesohepar*, to the pericardial sac by the *coronary ligament*, and to the mid-ventral line of the body wall by the *falciform ligament*. The liver is divided into *right* and *left lobes*. The left lobe is much the larger and is subdivided by a cleft into *anterior* and *posterior* parts. The right lobe is undivided. The *middle* lobe of the liver is concealed from ventral view by the other lobes but is revealed when they are raised as a small projection extending between the right lobe and the posterior part of the left lobe. In the deep fissure between right and left lobes of the liver is situated the round green *gall bladder*.

The liver secretes a fluid, the *bile*, which is stored up in the gall bladder. The bile aids in the digestion of fats. The liver also stores up digested food which is not immediately needed by the body and is further the chief site of formation of the nitrogenous wastes.

6. Pancreas.—Turn the lobes of the liver forward and study the *pancreas*. It is an irregular branched yellowish gland lying in the gastro-hepato-duodenal ligament. It sends down tail-like processes to the duodenum and a *hepatic process* along the dorsal surface of the liver where it extends almost to the gall bladder. The pancreas secretes into the duodenum a digestive fluid containing enzymes which perform most of the work of digestion. It is also a gland of internal secretion.

7. Bile duct.—The *common bile duct* runs through the pancreas and enters the duodenum by one of the tail-like processes mentioned above, the one farthest away from the pylorus. Dissect away the substance of the pancreas and find the white bile duct, about the size of a sewing thread in the leopard frog, considerably larger in the bull frog. Trace the duct forward through the pancreas toward the liver, picking away the substance of the pancreas. Small *hepatic ducts* will be seen to enter the common bile duct from the posterior part of the left lobe of the liver. Follow the bile duct toward the gall bladder and note additional hepatic ducts from the adjacent liver and *cystic* ducts from the gall bladder emptying into the common bile duct. As the bile duct passes through the pancreas it receives *pancreatic* ducts from this gland but these

are impractical to locate. It will be seen that the common bile duct conveys both the bile and the pancreatic secretion into the duodenum.

8. Large intestine.—Trace the ileum posteriorly and note where it widens suddenly into the *large intestine*. The latter is supported by a portion of the same mesentery that attaches the rest of the digestive tract and called here the *mesorectum*. In the mesorectum is situated the spleen. Observe the *urinary bladder* at the posterior end of the coelome. Note its attachment to the median ventral line by the *median ligament* of the bladder; to the large intestine by the *rectovesical ligament;* and to the lateral body wall on each side by *lateral ligaments* of the bladder.

The large intestine passes through the bony ring formed by the pelvic girdle and terminates at the anus. To trace its course cut through the pelvic girdle with a strong scissors slightly to the left of the mid-ventral line and spread the cut edges apart. Trace the large intestine into the anus. Note the place where the stalk of the urinary bladder enters the ventral wall of the intestine. This part of the large intestine is called the *cloaca* because it is not merely a part of the digestive tract but also serves the urogenital system as will be seen shortly.

Draw the entire digestive tract, showing all of its parts, with the lobes of the liver turned forward. Enter the common bile duct in proper position in the pancreas and draw in the ducts it receives from liver and gall bladder as far as you were able to find them.

B. THE UROGENITAL SYSTEM

This system consists of the kidneys and reproductive organs and their ducts.

Remove the digestive tract by cutting across esophagus and ileum, leaving the large intestine in place. Strip off peritoneum wherever necessary to expose the urogenital system.

1. Male urogenital system.—Each testis is an oval body attached by a mesentery, the *mesorchium*, to the ventral surface of the adjacent kidney. From each testis spring several delicate ducts, the *vasa efferentia* or *efferent ducts*, which run in the mesorchium and penetrate the kidney. They can generally be seen by holding up the mesorchium to the light. The vasa efferentia are really extensions of the tubules of the kidney which grow out into the testis. The vasa efferentia after entering the kidney eventually connect with the mesonephric duct by a route which differs in different species of frogs.

The kidneys are the two flat reddish organs located in the cisterna magna. Each has a duct, the *mesonephric duct*, which will be found along its lateral margin. Find this duct near the posterior end of the kidney and trace it to the cloaca, breaking through the peritoneum where necessary. As noted in chapter i, there is in the male leopard frog (but not in the bull frog) a delicate duct alongside and parallel to the lateral edge of each kidney, which corresponds to the oviduct of the female. The mesonephric duct is adherent to its dorsal surface and both pass together to the cloaca.

Cut open the cloaca by a longitudinal slit to the left of the midventral line. Note the large opening of the urinary bladder in its ventral wall. The two openings of the mesonephric ducts are in the dorsal wall opposite the opening of the bladder but are not very easy to locate.

Make a drawing of the male urogenital system with cloaca cut open showing all parts that you saw.

It is characteristic of Amphibia and many fishes that the ducts of the kidneys carry to the exterior the spermatozoa as well as the urine. This is not the case in reptiles, birds, and mammals. The mesonephric ducts of the males of these animals are reserved solely as sexual ducts and their kidneys (which do not exactly correspond to those of the frog) are provided with a new pair of ducts, the ureters, not present in the frog.

2. Female urogenital system.—The ovaries were previously identified. Each is fastened to the dorsal wall of the coelome by a mesentery, the *mesovarium*, which is well developed when the ovary is mature. The *oviducts* are a pair of convoluted tubes, one to the lateral side of each ovary. Each is supported by a mesentery, the *mesotubarium*. Trace one of the oviducts forward and note that it begins at the anterior end of the coelome near the base of the lung. At this place it is fastened to the peritoneum and opens into the coelome by an opening termed the *ostium*. Try to find this. Trace the oviduct posteriorly. At about the level of the posterior end of the kidney it passes through the peritoneum into the cisterna magna where it widens into a large thin-walled sac, the *uterus*. The uterus proceeds to the cloaca.

The kidneys and mesonephric ducts are as described above for the male. Note that the mesonephric ducts are adherent to the dorsal surface of the uteri. Trace both of them to the cloaca. Cut open the cloaca by a longitudinal slit slightly to the left of the mid-ventral line. In the ventral wall of the cloaca locate the large opening of the urinary bladder. Opposite this on the dorsal wall find the two *papillae* where the uteri terminate. The openings of the mesonephric ducts are small pores just posterior to these papillae.

Make a drawing of the female urogenital system with cloaca opened showing all parts that you have seen.

In the female, unlike the male, the reproductive organs have no connection with the kidneys. Further, the oviducts are not connected to the ovaries. The eggs escape from the ovaries by rupture and are carried by ciliary currents to the ostium of the oviducts down which they pass. They sojourn for a time in the uterus. For details of the breeding process in frogs, texts should be consulted. The lack of relation of ovaries to either kidneys or oviducts is universal throughout the vertebrates.

C. THE RESPIRATORY SYSTEM

In land vertebrates this system consists of a pair of lungs and the passages leading from the outside to the lungs. The main parts of this system were previously identified.

The *larynx* is a chamber between the glottis and the lungs, situated just in front of the heart. To reveal it, dissect away all muscles from the under surface of the lower jaw so as to expose the body of the hyoid cartilage. Note the two *thyroid processes* of the hyoid which extend posteriorly. The larynx is inclosed between these two processes. The walls of the larynx are supported by a complicated arrangement of cartilages. Dissect posteriorly from the larynx cutting through blood vessels or other structures until you have exposed the connection of the lungs with the larynx. Make a longitudinal slit

in the ventral wall of the larynx, spread the sides of the slit apart, and look within for the *vocal cords,* a pair of longitudinal folds of the walls of the larynx. Find the openings of the lungs into the latero-posterior walls of the larynx, insert one blade of a scissors into one of the openings, and slit open the wall of the lung. The inner surface of the lung is composed of a network of ridges which divide the wall into a large number of small chambers, called *alveoli* or *air sacs*. Blood vessels run along the ridges and break up into an intricate network of capillaries in the walls of the air sacs.

Draw the dissection.

D. THE CIRCULATORY SYSTEM: THE VENOUS SYSTEM

In chapter i it was explained that there are two circulatory systems in vertebrates, the blood-vascular system and the lymphatic system. As commonly used, however, the term circulatory system refers only to the blood-vascular system. This system is composed of the heart, veins, arteries, and capillaries, but the last of these cannot, of course, be seen with the naked eye. The *venous system* includes all of those blood vessels in which the blood flows *toward* the heart; it *cannot* be defined on the basis of the kind of blood contained in the vessels.

For the study of the circulatory system a fresh frog, probably injected, will be supplied. Open it up in the usual way to the left of the median line, but be very careful not to cut any blood vessels, especially near the heart. Be very cautious in spreading the body walls apart near the heart, so as not to injure the main veins. The ventral abdominal vein in the mid-ventral line *must be preserved* and should be separated from the body wall before spreading out the walls.

Make a large outline drawing of the frog and its chief organs and on this outline enter the veins as you find them, putting them in proper relation to the organs. More than one drawing may be necessary. Put in only those veins which you found.

1. The systemic veins.—*Systemic veins* are those which open into the heart, returning blood from the body directly to the heart. Remove the pericardial sac from the heart, and turn the heart forward so as to expose the sinus venosus. Three large veins contribute to the sinus venosus: the *posterior vena cava,* or *postcaval* vein, which emerges from the liver (receiving a large vein on each side from the liver so that apparently three veins are present) and enters the median posterior wall of the sinus; and the two *anterior venae cavae,* or *precaval* veins, one opening into each lateral wall of the sinus.

a) *The precaval vein:* Trace one of the precaval veins (since both have identical branches). It extends laterally from the heart along the border of the auricle, and about 1 cm. away from the heart passes through the pleuroperitoneal membrane. Directly outside of this membrane it forks simultaneously into three branches. The most anterior one of these is the *external jugular;* it passes straight anteriorly into the muscles of the floor of the mouth, from which, together with the tongue, hyoid, etc., it receives venous blood through numerous small branches. The middle of the three branches of the precaval is the *innominate;* it passes laterally and then turns abruptly dorsally, receiving as it turns a small *subscapular* vein from the muscles of the shoulder. The main vein bevond the entrance of this branch is known as the *internal jugu-*

lar; it descends straight into the hollow between the fore limb and the larynx, and disappears dorsally where it collects blood from the brain and spinal cord, from the roof of the mouth and from a number of muscles. The third and posterior branch of the precaval is the *subclavian.* It is a large vein passing directly laterally to the base of the fore limb where it receives the *brachial* vein, carrying venous blood out of the fore limb. Just beyond this point the vein, now called *cutaneous,* or *musculo-cutaneous,* turns abruptly and runs straight posteriorly along the muscle of the ventro-lateral body wall (pectoral muscle). It then bends sharply dorsally and passes to the skin by way of the partition between the abdominal and lateral lymph sacs. It runs anteriorly under the skin forward to the nares, collecting venous blood from the skin of the entire dorsal side.

b) The postcaval vein: Trace the postcaval posteriorly from the sinus venosus into the liver. Where it emerges from the liver substance it receives two large *hepatic* veins, one from the right lobe, the other from the left lobe of the liver. Turn the lobes of the liver to the right and find where the postcaval enters the middle lobe from behind. Trace it posteriorly. It originates between the two kidneys, from which it receives a number of *renal* veins as well as veins from the reproductive organs and fat bodies.

2. **The hepatic portal system.**—A *portal system* is one in which the venous blood does not return directly to the heart but enters a system of capillaries in some organ from which the venous blood is then collected by a systemic vein. There are two portal systems in the frog, the *hepatic* portal system in which the interposed capillaries are in the liver, and the *renal* portal system where they are in the kidney. Spread out the liver with its lobes turned forward so that the pancreas is visible. Find a large vein ascending through the substance of the pancreas and forking on the dorsal surface of the liver. This is the *hepatic portal* vein which breaks up into a system of capillaries in the liver. The left fork of the hepatic portal vein sinks into the substance of the posterior part of the left lobe of the liver. The right fork connects with the ventral abdominal vein which also forks and then penetrates the liver. Trace the hepatic portal vein posteriorly and note that it is formed by the union of veins from the stomach, pancreas, spleen, and small and large intestines. All of the blood from the digestive tract, therefore, enters a capillary system in the liver, from which it flows into the postcaval vein by way of the hepatic veins. What is the purpose of this arrangement?

3. **The renal portal system.**—Expose the kidneys and find along the outer lateral edge of each a conspicuous vein, the *renal portal* vein, which enters the substance of the kidney. Trace the renal portal vein posteriorly. At about the middle of the kidney it receives a vein, the *dorso-lumbar* vein, from the muscles of the back. Posterior to the kidney it is seen to be formed from two veins which come from the leg. The outer of the two veins is the *femoral,* the inner, the *sciatic.* At the point where each femoral vein enters the coelome, it gives off a branch, the *pelvic* vein, which runs along the posterior wall of the coelome to the median line where it joins the other pelvic. This union of the two pelvic veins produces the abdominal vein, which joins the hepatic portal vein as described above. The abdominal vein is thus a connection between the renal and hepatic portal systems, and blood from the hind legs may return to the heart either by

way of the kidneys or by way of the liver. In the kidneys the renal portal vein breaks up into capillaries, from which the blood is collected by the postcaval vein.

4. The pulmonary veins.—From each lung a *pulmonary* vein passes dorsal to the sinus venosus and enters the left auricle. These veins are sometimes difficult to locate. Turn the heart forward holding it down with the finger so that it will be stretched. Pull away with a forceps the coronary ligament of the liver so as to expose the lungs. A short straight vessel will be found running from the inner border of the base of each lung obliquely toward the sinus venosus. These two pulmonary veins join just dorsal to the beginning of the left precaval vein and penetrate the left auricle at that point.

E. THE CIRCULATORY SYSTEM: THE ARTERIAL SYSTEM

The *arterial system* consists of vessels in which the blood flows *away* from the heart. It cannot be defined on the basis of the kind of blood which it contains.

Make an outline drawing as directed under the venous system and on this enter the arteries as you find them, showing their relations to the parts of the animal. Omit those you could not locate.

The conus arteriosus starts from the base of the right side of the ventricle, passes obliquely across the auricles, and divides near the anterior border of the auricles into two vessels which turn respectively to the right and left. Each branch is called a *truncus arteriosus* and each gives rise shortly to three arteries, which are designated as *aortic* or *arterial arches*. To find this division into three, carefully pick off the connective tissue, etc., from the surface of one truncus, and follow it away from the heart. The most anterior of the three arches is the *carotid arch;* the middle one is the *systemic arch;* the most posterior, the *pulmo-cutaneous arch*. The pulmo-cutaneous arch generally branches off before the other two.

1. The carotid arch.—This vessel passes forward and soon divides into two branches, a medial small *external carotid,* and a lateral larger *internal carotid*. Just at the place of division, or situated on the internal carotid, is an enlargement, the *carotid gland,* presenting a blackish appearance owing to the presence of pigment cells. The internal carotid supplies the roof of the mouth, eye, brain, and spinal cord. The external carotid (or *lingual*) supplies the tongue, thyroid, and various muscles. Trace the branches of the carotid arch as far as practicable.

2. The pulmo-cutaneous arch.—Trace this out from the truncus arteriosus, and note that it soon divides into two branches. One of these, the *pulmonary,* is short, and runs directly to the lung. The other, the *cutaneous,* passes outward and forward, crossing the systemic arch, and then by a sharp dorsal turn disappears in front of the shoulder. Turn the frog dorsal side up, slit up the skin of the back, and deflect it. Find where the cutaneous artery emerges just in front of the suprascapula, and note its branches on the skin.

3. The systemic arch.—The systemic arch passes laterally and forward, and seems to disappear in the mass of muscles. Dissect away these muscles and follow its course. It runs forward alongside the internal carotid, then bends dorsally and turns backward dorsal to the esophagus. Just after it has made this posterior turn, the systemic arch gives rise to the following branches practically simultaneously: The small

esophageal arteries, which supply the esophagus; the *subclavian*, which passes laterally to the fore-leg; and the *occipito-vertebral*, which passes dorsally and promptly divides into an anterior branch, the *occipital*, and a posterior branch, the *vertebral*. Both of these can be readily followed in well-injected specimens. Turn the frog dorsal side up, and find the occipital artery emerging just under the anterior border of the suprascapula. Follow it along the head, noting a branch above the eye to the anterior parts of the head, and one behind the eye for the upper and lower jaws. Remove all muscles from the vertebral column, and note the intricate branches of the vertebral artery to the vertebrae.

The two systemic arches from the two sides of the body now converge and unite to form a single large vessel, the *dorsal aorta*. The dorsal aorta lies in the cisterna magna, above the kidneys, and the pleuroperitoneum must be broken through in the usual way to one side of one of the kidneys in order to follow this vessel. At the point of union of the systemic arches, the large *cocliaco-mesenteric* artery arises. Trace the branches of this artery. It divides into a *coeliac*, which supplies the stomach, pancreas, and liver (*right* and *left gastric* arteries, and *hepatic* artery), and an *anterior mesenteric*, which gives off a short branch to the spleen (*lienal* artery), a number of branches to the small intestine (*intestinal* arteries), and one or more branches to the large intestine (*anterior haemorrhoidal* arteries).

Posterior to the origin of the coeliaco-mesenteric, the dorsal aorta gives rise to a number of small paired *urogenital* arteries, which supply mainly the kidneys, but branch also to the fat bodies, and reproductive organs and their ducts. In the same region, one to four pairs of small *lumbar* arteries arise from the aorta and pass to the dorsal body wall.

The dorsal aorta next gives off a single median branch, the *posterior mesenteric*, to the large intestine, and shortly after this bifurcates into two large arteries, the *common iliac* arteries. Each common iliac soon gives rise to two branches. One of these, the *epigastrico-vesical* artery, branches almost immediately into an *epigastric* artery for the muscles of the lateral and ventral abdominal walls, and a *recto-vesical* artery for the urinary bladder and the rectum. The other is a small artery to the urogenital ducts. The iliac then supplies a small *femoral* artery for the muscles of the thigh and continues down the leg as a large vessel, the *sciatic* artery.

Be able to trace the blood from any part to any other part.

F. THE CIRCULATORY SYSTEM: THE STRUCTURE OF THE HEART

The four parts of the heart, the sinus venosus, the auricles, the ventricle, and the conus arteriosus, have already been noted. Draw the heart from the side; show these parts.

The dissection of the heart should not be attempted except upon a well-preserved, uninjured heart. The heart of injected specimens cannot be used. With a fine scissors remove the ventral wall of the ventricle, the ventral wall of each auricle, and the ventral wall of the conus arteriosus as far as the working of the trunci arteriosi. If desirable, the heart may be removed from the body by cutting through the aortic arches and the sinus venosus; then mount the heart under water in a small wax-bottomed dish. Note the thick

spongy walls of the ventricle and its small central cavity. Find the openings of the two auricles into the ventricle. The walls of the auricles are quite thin and the right one is considerably larger than the left. The large opening of the sinus venosus into the right auricle is readily found. The conus arteriosus contains a conspicuous structure, the *spiral valve,* which almost fills its cavity. It is a fold, bent slightly into an **S**-shape, extending lengthwise in the conus, its dorsal edge attached along the entire extent of the dorsal wall of the conus, its ventral margin free. In the cross-section of the truncus arteriosus observe the two partitions which divide it into three channels, one for each aortic arch. The ventral channel leads to the carotid arch, and the middle one to the systemic arch; the beginnings of these two channels are in the conus arteriosus in front of the termination of the spiral valve, so that the blood to reach them must flow over the cuplike widened anterior end of the valve. The dorsal channel of the truncus arteriosus passes into the pulmo-cutaneous arch; it starts farther down in the conus below the anterior end of the spiral valve, so that blood reaches it by flowing over the free ventral edge of the valve. From texts understand the function of the spiral valve and the partitions in the truncus arteriosus in directing the venous blood into the pulmo-cutaneous arch, mixed blood into the systemic arches, and the arterial blood into the carotid arch.

Draw the dissection from the ventral side.

G. THE NERVOUS SYSTEM

The nervous system of vertebrates consists of the brain, spinal cord, and sympathetic system, with all of the nerves coming to and from them.

Remove the skin and clean way all muscles from the median dorsal region of the animal. With a scalpel make a cut passing through both external nares and cautiously continue this cut posteriorly stripping or pulling off the bones of the roof of the skull Be careful not to jab into the soft brain beneath. Continue posteriorly cutting off the dorsal parts of the vertebrae. Brain and spinal cord should be well exposed before proceeding.

1. **Dorsal aspect of the brain.**—The brain is covered with a pigmented membrane, the *pia mater,* which is particularly abundant in the posterior part of the brain, where it is very vascular and fills a triangular cavity, the *fourth ventricle.* The pia mater should be removed from the fourth ventricle.

The most anterior part of the brain comprises a pair of rounded *olfactory lobes,* which are separated from each other by a faint median groove. Each olfactory lobe continues forward to the nasal sac as the *olfactory nerve.* The posterior boundary of the olfactory lobes is marked by a transverse groove which separates them from the next part of the brain, a pair of long oval bodies, the *cerebral hemispheres.* Just behind the cerebral hemispheres is a depressed region, the *diencephalon* or *thalamencephalon,* from which a delicate stalklike body, the *pineal body,* ascends dorsally to the brow spot. The pineal body is almost always torn off in removing the skull and hence cannot be seen. Posterior to the diencephalon are the two rounded *optic lobes.* Just behind them and forming the anterior wall of the triangular cavity of the fourth ventricle is the *cerebellum.* This is much smaller in the frog than in most other vertebrates. The region of the brain posterior to the cerebellum, and forming the floor and lateral walls of the

fourth ventricle is known as the *medulla oblongata.* It narrows posteriorly until it becomes of the same width as the spinal cord with which it is continuous.

2. **The cranial nerves.**—Ten pairs of nerves spring from the lateral and ventral surfaces of the brain but most of these cannot be found unless very great care is exercised in dissecting. The first pair of cranial nerves, the olfactory, has already been noted arising from the olfactory lobes. The second pair of nerves, the *optic* nerves, springs from the ventral surface of the diencephalon, and may be seen by gently pushing this part of the brain to one side. Each penetrates the adjacent eyeball where it enters the retina. The third, fourth, and sixth nerves are motor nerves to the muscles of the eyeball and are too small to be found. The fifth, seventh, and eighth nerves arise close together from the side of the anterior end of the medulla, and may usually be seen by gently pressing back this region. The ninth and tenth arise together from the side of the medulla a short distance behind the eighth.

3. **The spinal cord.**—The spinal cord is continuous with the medulla oblongata and occupies a cavity within the vertebral column known as the *neural canal.* Posteriorly the spinal cord tapers into a fine thread, the *filum terminale,* which occupies the cavity of the urostyle and can be seen by cutting off the dorsal half of the urostyle. The spinal cord is slightly enlarged opposite the fore limb (*brachial enlargement*) and again anterior to the filum terminale (*sciatic enlargement*). These swellings are, of course, caused by the origin of the nerves to the limbs at those regions. In the median dorsal line of the cord is a longitudinal groove, the *dorsal fissure.* In a well-dissected specimen, the *dorsal roots* of the *spinal nerves* may be seen arising at regular intervals from the dorso-lateral region of the cord. The most posterior of these roots run within the neural canal for a little distance alongside the spinal cord before passing out of the vertebral column.

Draw the brain and cord from the dorsal side.

4. **Ventral aspect of the brain.**—Cut across the cord back of the *medulla oblongata,* and carefully remove the brain, leaving the cord in place. In removing the brain note and cut through the more conspicuous of the cranial nerves. Study the ventral surface of the brain and identify the parts already noted on the dorsal side. On the ventral side of the diencephalon observe the crossing of the optic nerves, after their origin from the diencephalon; this crossing is called the *optic chiasma.* Just behind the optic chiasma is a bilobed extension of the floor of the diencephalon, called the *inferior lobes* or *infundibulum;* and to this is attached a rounded glandular body, the *hypophysis,* which fits into a depression in the floor of the skull, and is usually, therefore, torn off in removing the brain. The hypophysis is a gland of internal secretion.

Draw the ventral surface of the brain.

5. **The ventricles of the brain.**—The brain like the spinal cord is hollow, its cavity being continuous with the central canal of the cord. The cavities of the brain are known as *ventricles.* Make a median sagittal section of the brain, float it under water, and identify the ventricles. The cavity of the medulla oblongata is the largest and most posterior of the ventricles; it is called the *fourth ventricle* and has a thin vascular roof which has already been removed. From the fourth ventricle a narrow passage, the *cerebral aqueduct,* extends forward below the optic lobes. Each optic lobe has a cavity, the

optic ventricle. The ventricle of the diencephalon which extends downward into the infundibulum is the *third* ventricle. The *first* and *second* ventricles, also called the *lateral* ventricles, are inside of the cerebral hemispheres, which should be cut open to see them. The narrow passage which connects the lateral with the third ventricles is known as the *interventricular foramen.*

6. The spinal nerves.—Turn the frog ventral side up, and remove all the viscera. Observe the spinal nerves passing out symmetrically from the sides of the vertebral column. They arise from the cord and leave the neural canal by way of openings (*intervertebral foramina*) between the vertebrae. There are ten pairs of spinal nerves, each of which divides immediately into a small *dorsal branch* and a larger *ventral branch.* It is the ventral branch which one sees running on the inside of the dorsal body wall. At the point of exit of each spinal nerve from the intervertebral foramen is a white mass, the *calcareous body,* which surrounds and conceals a *ganglion.*

Identify and observe the course of each of the spinal nerves. The first is quite small; it arises between the first and second vertebrae and innervates the tongue and muscles of the hyoid. The second is a large stout nerve which innervates the muscles of the fore limb. It is joined by branches of the first and third spinal nerves, and all of these together form a network which is called the *brachial plexus.* The presence of such a plexus indicates the compound origin of the muscles of the limb. The fourth, fifth, and sixth nerves are small and pass somewhat obliquely backward to supply the skin and muscles of the body wall. The seventh, eighth, ninth, and tenth nerves arise close together, run almost directly backward, and are united with one another by cross branches to form the *sciatic plexus* from which nerves go to the muscles and skin of the hind limbs. The small tenth nerve arises from the urostyle, and innervates mainly the cloaca and urinary bladder.

Draw the spinal nerves as seen on the dorsal body wall.

7. **The roots of the spinal nerves.**—Select one of the largest spinal nerves (as the eighth), and trace it carefully into the vertebral column, cutting away the vertebrae. Pull off the calcareous body and find within it a small brown object, the *dorsal* or *spinal ganglion.* (The term "ganglion" means a mass of nerve cell bodies lying outside of the brain and spinal cord.) Tracing the nerve farther in toward the cord note that it divides into two branches or *roots,* one of which is attached to the dorso-lateral region of the cord, the other to the ventro-lateral region. These roots are designated as the *dorsal* and *ventral roots.* The dorsal root springs from the dorsal column of the gray matter of the cord; it carries sensory fibers which arise from the nerve cells located in the dorsal ganglion. The ventral root takes its origin from the ventral column of the gray matter, from the large motor cells which have already been seen in that location, and carries motor fibers to the muscles. The two roots meet just beyond the spinal ganglion, which is on the dorsal root, and the spinal nerve thus formed soon divides into dorsal and ventral branches or *rami,* as noted in the preceding section. Each ramus carries both sensory and motor fibers and supplies both skin and muscles; and the ventral ramus is further connected by the *ramus communicans* with the sympathetic system.

Make a diagram to show the origin of a spinal nerve from the cord.

H. THE SKELETON

For this work dried mounted skeletons will be provided. (The student may prepare a skeleton as follows: Remove all possible flesh and organs from a freshly killed frog, and dip what remains from time to time in hot water, brushing away the remaining flesh until the bones are cleaned. Too liberal use of hot water or boiling will cause the bones to fall apart.)

1. General considerations on the skeleton.—The skeleton, or hard parts of the body, is generally of two kinds: the external skeleton, or *exoskeleton,* and the internal skeleton, or *endoskeleton.* The exoskeleton covers and protects the body and is derived from the skin. Examples are scales, feathers, hair. The frog has no exoskeleton except such as has become fused to the endoskeleton (see below). The endoskeleton is the internal framework of the body, and is composed of cartilage in the embryo which becomes partially converted into bone in the adult frog. Such bone formed in cartilage is known as *cartilage bone.* Investigation shows that not all the bones of the frog's skeleton have arisen in this way but some have appeared in connective tissue without passing through a cartilage stage. Such bones are called *membrane bones, dermal bones,* or *investing bones.* They are produced from the dermis of the skin, and are, therefore, not really endoskeleton, but part of the exoskeleton. In the course of evolution these bones, originally scales, sank into the body and attached themselves so closely to the endoskeleton that they are usually treated in textbooks as parts of the endoskeleton. The clavicle and the superficial bones of the skull and jaws of the frog are membrane bones; all other bones of the skeleton are cartilage bones.

The skeleton may be divided into an *axial* part including the *skull,* the *visceral skeleton,* and the *vertebral column;* and an *appendicular* part consisting of the bones of the limbs, the *girdles,* and the *sternum* or *breastbone.*

2. The vertebral column.—The anterior half of the vertebral column of the frog consists of nine bony rings, the *vertebrae;* the posterior half is an elongated slender bone, the *urostyle.* Obtain some isolated vertebrae and identify the following parts:

a) The *neural canal* is the cavity in the vertebra.

b) The *centrum* or *body* is the thickened part of the vertebra ventral to the neural canal. It articulates with the centra before and behind it by a ball and socket arrangement.

c) The *neural arch* is that portion which arches dorsally over the neural canal.

d) The *neural spine* is a sharp dorsal projection from the center of the neural arch.

e) The *transverse processes* are the conspicuous lateral projections extending horizontally outward from the place of junction of centrum and neural arch.

f) The *zygapophyses* are anterior and posterior projections from the neural arch, one in front of and one behind the place of origin of each transverse process. They link successive vertebrae together.

The first vertebra, or *atlas,* differs from the others in the absence of the transverse processes and the presence of special articulating surfaces for holding the skull. Between the atlas and the skull is a gap which permits the operation of pithing. The transverse processes of the last vertebra articulate with the pelvic girdle, and this vertebra is hence called the *sacral* vertebra.

3. The skull and the visceral skeleton.—The visceral skeleton includes the upper and lower jaws, the hyoid apparatus, and the cartilages of the larynx. It is so called because originally all of these structures were paired semicircular cartilages, used to support the gills. Since gills are part of the walls of the alimentary canal, the appropriateness of the term "visceral skeleton" becomes apparent. Six such cartilaginous hoops are present in the frog tadpole, of which the first becomes in the adult frog the basis of upper and lower jaws, while the remaining ones undergo remarkable transformations into the hyoid and laryngeal cartilages. The laryngeal cartilages will not be studied.

The original cartilage skull, forming a case to inclose the brain, fused in front with the *olfactory capsules* containing the organ of smell, behind with the *otic capsules* containing the ears, and below with the cartilaginous bars which formed the upper jaw while the similar bars of the lower jaw remained separate and formed a joint with the skull. Part of the cartilage of these structures persists in the adult skull, part is converted into cartilage bones, and both of these are partly concealed by a superficial covering of membrane bones.

a) Dorsal aspect of the skull and upper jaw.—Examine the dried skull and note that the membrane bones can be distinguished easily from the cartilage bones by their lighter color, smoother surfaces, thin and flat shapes, and more superficial position. The cartilage has, of course, disappeared in a dried skull. The skull proper forms the central region, while the upper jaw, which is fused to the skull, appears as an arch on each side and in front of the skull. A large gap, the *orbit,* which holds the eye, is thus left between skull and jaw on each side.

The bones of the dorsal side of the skull proper are:

(1) *Nasal* bones, two triangular membrane bones just behind the external nares.

(2) *Sphenethmoid* bone, a single ring-shaped cartilage bone in the median line behind the nasals.

(3) *Frontoparietal* bones, two long flat membrane bones posterior to the sphenethmoid. In other animals the anterior *frontal* portion is separate from the posterior *parietal* portion of these bones.

(4) *Exoccipital* bones, the two cartilage bones forming the posterior extremity of the skull. They surround a large opening, the *foramen magnum,* through which the medulla oblongata passes to become continuous with the spinal cord. Ventrally they bear two rounded prominences, the *occipital condyles,* by which the skull articulates with the atlas.

(5) *Pro-otic* bones, the cartilage bones extending laterally from the posterior end of the frontoparietals. They are ossified in the otic capsule and hence inclose the ear.

The bones of the upper jaw or *maxillary arch* are:

(1) *Premaxilla,* a pair of small membrane bones in front of the external nares.

(2) *Maxilla,* the long slender membrane bone forming the greater part of the sides of the jaw. Premaxillae and maxillae bear teeth.

(3) *Quadratojugal,* the short slender membrane bone behind the maxilla, not bearing teeth.

(4) *Squamosal,* the curious **T**-shaped membrane bone extending from the posterior end of the quadratojugal to the pro-otic.

(5) *Pterygoid,* a three-rayed cartilage bone under the squamosal but visible from the dorsal side.

(6) The *quadrate* cartilage lies between the squamosal and the pterygoid and is the place of attachment of the lower jaw to the skull.

b) Ventral aspect of the skull. The ventral bones of the skull are:

(1) *Vomer,* a pair of membrane bones behind the premaxillae, forming the floor of the olfactory capsules and bearing teeth.

(2) *Palatine,* two slender cartilage bones extending laterally from just behind the vomerine teeth to the maxillae.

(3) *Parasphenoid,* a single long dagger-shaped membrane bone on the ventral surface of the skull, its lateral posterior processes underlying the auditory capsules. The point of the dagger underlies the ventral side of the sphenethmoid ring.

c) The bones of the lower jaw or *mandibular arch.* These are:

(1) *Mentomeckelian* bones, two small cartilage bones at the tip of the lower jaw. They are ossified in the original cartilage bars (Meckel's cartilage) which were the lower jaw of the tadpole.

(2) *Dentary,* a short membrane bone behind the preceding on the outer surface of the jaw.

(3) *Angulosplenial,* the long slender membrane bone forming the greater part of the jaw. Its anterior end is under the dentary. Its outer surface behind the dentary is grooved. In this groove is located in life Meckel's cartilage, which articulates with the quadrate cartilages of the skull.

d) Hyoid apparatus. This portion of the skeleton is usually lacking in dried material. Most of its parts have already been seen during the dissection of the frog and should be examined again and further exposed in your preserved specimen.

(1) The *body* of the hyoid is the flat plate of cartilage in the floor of the buccal cavity. Its concave anterior margin receives the base of the tongue. Its lateral anterior corners have short *alary* processes, and its posterior corners *postero-lateral* processes.

(2) The *anterior horns* or *cornua* of the hyoid are the long, slender rods which curve back from the anterior margin of the body to the pro-otic bones of the skull.

(3) The *thyroid* processes or *posterior horns* are the two processes which diverge from the posterior margin inclosing the laryngeal chamber between them. They are ossified while the other parts of the hyoid are cartilaginous.

(4) The *columella,* the small slender bone in the middle ear, one end of which is attached near the middle of the tympanic membrane, is probably a part of the hyoid apparatus.

4. Bones of the pectoral girdle.—The pectoral girdle forms a bony arch for the support of the fore limbs. It is incomplete dorsally and has no connection with the vertebral column. Ventrally its two halves are united by the interposition of the sternum. Each half of the girdle consists of:

a) *Suprascapula,* the most dorsal bone, large and flat with a cartilage along its free dorsal border.

b) *Scapula,* the bone ventral to the preceding and containing a cup-shaped cavity, the *glenoid fossa,* in which the long bone of the upper arm is inserted.

c) *Clavicle,* the anterior bone of the two which compose the ventral aspect of the girdle. It is a membrane bone, the only one in the skeleton outside of the skull and jaws.

d) *Procoracoid,* the cartilage which is covered over by the clavicle and which fails to ossify because its function is usurped by the clavicle.

e) *Coracoid,* the posterior of the two ventral bones. It takes part in the glenoid fossa.

5. The sternum.—The sternum is a chain of bones and cartilages between the two ventral ends of the halves of the pectoral girdle.

a) *Episternum,* the rounded cartilage forming the anterior extremity of the sternum.

b) *Omosternum,* the bone behind the preceding.

c) *Epicoracoids,* the cartilages between the medial ends of the coracoid bones.

d) *Sternum* proper or *mesosternum,* bony rod behind the coracoids.

e) *Xiphisternum,* terminal rounded cartilage.

6. Bones of the pelvic girdle.—This is the bony girdle which supports the hind limbs. It is complete dorsally, forming a joint with the transverse processes of the last vertebra. The bones of each half of the pelvic girdle are:

a) *Ilium,* the long scythe-shaped dorsal bone which extends forward parallel to the urostyle to articulate with the transverse processes of the sacral vertebra.

b) *Pubis,* the anterior portion of the semicircular crest which projects ventrally from that part of the pelvic girdle which lies medially between the heads of the two long thigh bones.

c) *Ischium,* the posterior portion of the crest.

The two pubes and the two ischia are completely fused in the median ventral line producing the projecting crest mentioned above. These unions are named the *pubic* and *ischial symphyses.* The cuplike cavity on each side of the girdle which receives the head of the thigh bone is called the *acetabulum.*

7. Bones of the limbs.—The skeleton of the fore and hind limbs is evidently built upon the same plan, and the bones evidently correspond. The two limbs will therefore be considered together.

a) The upper part of each limb consists of a long bone. In the fore limb this is the *humerus;* in the hind limb, the *femur.* The humerus bears a conspicuous crest, the *deltoid ridge,* so named because the deltoid muscle is inserted there.

b) The next section of the limb is generally composed of two bones, but in the frog these two are in the case of both limbs fused into one. This is the *radio-ulna* in the fore limb, *tibio-fibula* in the hind limb. A longitudinal groove along the center of both surfaces of each of these bones indicates the place of fusion of the two originally separate components, the *radius* and *ulna* in the forearm, the *tibia* and *fibula* in the shank. Radius and tibia correspond and are on the thumb side (*preaxial* side) of the limb; ulna and fibula are on the little finger side (*postaxial*).

c) Wrist and ankle constitute the next section of the limbs. The wrist or *carpus* consists of six small bones in two rows. These are generally difficult to make out in ordinary preparations of the skeleton. The ankle or *tarsus* is unusually elongated in

the frog and consists chiefly of two relatively large bones, the *astragalus* on the preaxial side and the *calcaneum* on the postaxial side. Between these and the foot, two or three minute bones occur.

d) The palm of the hand and sole of the foot each consist of five slender diverging bones, the *metacarpals* and *metatarsals*, respectively. The first metacarpal is rudimentary.

e) The fingers and toes are supported by small bones, called *phalanges*.

I. THE MUSCULAR SYSTEM

The frog has two types of muscles, the *involuntary* muscles found in the viscera and the *voluntary* ones attached either directly or indirectly to the skeleton. The arrangement of the muscles of the viscera has already been seen in the study of the microscopic structure of the organs. They are commonly arranged in cylindrical tubes, in which the fibers run in either a circular or a longitudinal direction. The voluntary muscles, on the other hand, have no such simple arrangement, and a study of each one is necessary to understand its action. The term "muscular system" generally refers to the voluntary muscles.

For the study of the muscles frogs which have been preserved for some time in formalin should be employed. Those in which the viscera have been dissected are usable for this purpose. Remove the skin completely.

1. Parts and relations of a muscle.—Examine the large *gastrocnemius* muscle on the back of the shank as an illustration of a typical muscle. Identify its parts as follows:

a) The *fascia* is the shining tough connective tissue membrane which incloses the muscle.

b) The *tendon* is the shining tough band or cord at each end of the muscle. It is produced by the extension and concentration of the fascia beyond the fleshy part. By means of tendons, muscles are firmly attached to bones, other tendons, or other fixed structures. Tendons may be of many shapes, depending upon the shape of the muscle of which they are a part.

c) The *belly* is the fleshy part of the muscle.

d) The *origin* is the more fixed point of attachment of the muscle, i.e., the part which does not move when the muscle contracts. The gastrocnemius has two points of origin, or *heads*, as they are often called, at the upper end of its (apparently) dorsal surface. The larger of these attaches the main mass of the muscle to a tendon which passes from the distal end of the femur to the upper end of the tibio-fibula; the smaller is a slender tendon which joins the general tendon passing over the knee. With a forceps loosen the other muscles from about the heads of the gastrocnemius and verify these points.

e) The *insertion* is the movable point of attachment of the muscle, i.e., the part which is moved by the action of the muscle. The lower end of the gastrocnemius tapers to a strong tendon (the famous *tendon of Achilles*), which passes over and is attached to the ankle bones and then becomes continuous with the broad *plantar* fascia which covers the sole of the foot.

f) The *action* of a muscle is a description of its function. The gastrocnemius through its insertion on the ankle can bend the entire leg below the knee up against the thigh (*flexion* of the leg); through its continuity with the plantar fascia it can straighten the foot (*extension* of the foot). Test these actions by pulling on the gastrocnemius.

2. General account of the muscles of the frog.—We shall undertake to study only the superficial and easily identifiable muscles, particularly those which are of interest in the physiology of the frog. Most of the following muscles have the same names, positions, and action as in man and other vertebrates, and the statements about them are hence of general application. No attempt is made to give all the details of the origin and insertion.

In studying muscles separate each one from its fellows as carefully as possible with a forceps or probe, and determine the origin, insertion, and general action of each.

a) *Muscles of the lower jaw:* Pull off all tissue between the eye and tympanic membrane. Pull off the tympanic membrane, identifying underneath it a circular cartilage, the *tympanic ring*, resting upon the squamosal bone. Between the tympanic ring and the eye a mass of muscles will be found passing to the lower jaw, on the inner side of the posterior end of the upper jaw. Remove the end of the upper jaw (quadratojugal bone) so as to reveal the complete course of these muscles. The following three may be readily identified:

(1) The *temporal* muscle arises from the side of the skull and passes down between the eye and the tympanic ring to be inserted on the posterior end of the lower jaw. Action, closes the mouth (*elevator* of the jaw).

(2) The *masseters* originate from the tympanic ring and adjacent bones and are inserted on the lower jaw behind the temporal. Action, same as preceding; also stretch the tympanic membrane.

(3) The *depressor mandibuli* is a muscle of the jaw located behind the tympanic ring. It arises from the tympanic ring and from the general fascia of the back (*dorsal fascia*) and passes to the extreme posterior tip of the mouth jaw, where it is fastened to Meckel's cartilage. Action, opens the mouth (*depressor* of the jaw) and stretches the tympanic membrane. Pull the lower jaw fully open to see more clearly the arrangement of these three muscles.

b) *Muscles of the dorsal side of the trunk:* This portion of the body is more or less covered by the dorsal fascia, a strong membrane fastened to the ilium bones, the neural arches of the vertebrae, and the skull, and furnishing a place of insertion of many muscles. Easily identifiable muscles of the back beginning behind the ear are:

(1) The *dorsalis scapulae* is the anterior half of the large triangular mass behind and partially covered by the depressor mandibuli. Origin, from the dorsal margin of the suprascapula; remaining course like the next.

(2) The *latissimus dorsi* is the posterior portion of the triangular mass. Origin, dorsal fascia; unites with the preceding to be inserted on the deltoid ridge of the humerus; action, raises the fore limb upward and backward (abduction of the limb).

(3) The *longissimus dorsi* is the long muscle extending from the anterior third of the urostyle forward to the skull. Remove the dorsal fascia and preceding muscles to

see its full course. It is attached at many places to the vertebrae. Action, raises the head and straightens the back.

(4) The *coccygeo-sacralis* runs diagonally from the urostyle just behind the insertion of the preceding muscle to the transverse process of the sacral vertebra. Pull off the remainder of the dorsal fascia to see it. Action, draws the back nearer the urostyle or vice versa when both muscles of the two sides act together, or turns the back to one side, when acting singly.

(5) The *coccygeo-iliacus* is the diagonal muscle parallel to and behind the preceding running from the posterior two-thirds of the urostyle to the ilium. Action, fixes the urostyle with respect to the pelvic girdle.

c) Muscles of the sides of the abdomen:

(1) The *external oblique* is the large muscle covering the sides of the abdomen extending from the dorsal fascia and the ilium to the linea alba. Its fibers run diagonally backward. Action, to support and reduce the abdominal cavity, and to cause exhalation in lung breathing.

(2) The *transverse* (including the *internal oblique* of other vertebrates) lies under the preceding, its fibers running diagonally forward. Strip off the external oblique cautiously to see it. Relations and function similar to preceding.

d) Muscles of the ventral side of the trunk:

(1) The *rectis abdominis* is the flat segmented muscle lying on both sides of the linea alba, extending from the pubic symphysis to the sternum. Action, supports the abdominal contents, and fixes the sternum in place.

(2) The *pectoral* muscle is the large muscle of the anterior ventral part of the trunk. It arises from the various parts of the sternum and from the lateral border of the fascia of the rectus abdominis muscle. It is inserted on the deltoid ridge of the humerus. Action, draws the arm toward the ventral side and leg (adductor of the arm) and expands the abdominal cavity.

e) Muscles of the floor of the mouth and the hyoid apparatus:

(1) The *mylohyoid* is a thin sheet of muscle running crosswise from one half of the lower jaw to the other along their whole extent. It forms the outermost layer of the buccal floor and its function is to raise this floor in the breathing movements.

(2) The *submental* muscle. Cut through the mylohyoid muscle in its median ventral line and deflect the two halves. Internal to it at the very anterior tip of the lower jaw is the small submental muscle. Its contraction pushes the sublingual tubercle upward against the premaxillary bones of the upper jaw and thereby closes the external nares in lung respiration.

(3) The *geniohyoid* muscle comprises the longitudinal bands revealed by the removal of the mylohyoid, consisting of medial and lateral portions arising internal to the submental muscle and lateral to it from the lower jaw, and extending to the posterolateral processes of the hyoid and the body of the hyoid as far as the forking of the thyroid processes. Action, pulls the hyoid apparatus powerfully forward, thus raising the floor of the buccal cavity in respiration; also helps to swallow, to open the mouth, to lower the tip of the jaw, thus opening the nares, and to move the tongue.

(4) The *sternohyoid* is a continuation forward of the rectus abdominis, extending

from the dorsal surface of the coracoid and clavicle to the body of the hyoid. It is readily seen when the pectoral girdle is lifted up. It and a small muscle lateral to it (*omohyoid*) exert a pull upon the body of the hyoid, causing it to bulge outward, and hence lower the floor of the buccal cavity in breathing. Their action is thus the opposite of that of the preceding muscles.

(5) The *petrohyoids* are several small muscles internal to the sterno- and omohyoids extending from the otic capsule to the sides of the hyoid apparatus. They raise the hyoid apparatus and hence the floor of the buccal cavity in respiration, acting in antagonism to the preceding muscle, and by their compressing effect upon the larynx and pharynx are of great importance in swallowing food and air.

f) Muscles of the tongue:

(1) The *hyoglossus* is the conspicuous muscle in the median ventral line of the throat internal to the geniohyoid. Each half of it originates at the posterior end of the thyroid process of the hyoid, extends forward covering this process, and meets its fellow where the processes spring from the body of the hyoid. The muscle thus formed runs forward in contact with the body of the hyoid up to the base of the tongue into which it disappears. It is the *retractor* of the tongue (draws it back into the mouth after use).

(2) The *genioglossus* is a small but thick muscle lying in front of the anterior end of the hyoglossus and originating from the lower jaw internal to the submental muscle. It is the *protractor* of the tongue (throws it forward).

g) Muscles of the thigh: The thigh presents dorsal and ventral surfaces and anterior and posterior margins, but these are not the original relations because the hind limb has been rotated 90° forward from the primitive vertebrate position, in which the limb extended straight out laterally from the body. This torsion has made the original anterior (*preaxial*) margin ventral and the original posterior (*postaxial*) margin dorsal. However, in order to simplify the following description, the same names will be applied as for other parts of the body, according to the apparent positions. Separate all of the muscles of the thigh from each other before proceeding.

(1) The *triceps femoris* is the great muscle which covers the whole anterior part of the thigh, its powerful tendon passing over the knee to the tibio-fibula. It has three origins or heads and consists of three parts. The ventral part (*vastus internus* or *crural*) arises from the borders of the acetabulum. The small middle portion (*tensor fasciae latae*) originates on the ilium and ends in the fascia (*fascia lata*), which covers the triceps femoris. The dorsal part is the *vastus externus* or *gluteus magnus* and arises from the side of the posterior end of the crest of the ilium. The triceps femoris is the great extensor of the shank, and also may draw the leg up against the body (*abduction*)

(2) The *iliacus* muscles are those which extend from a considerable part of the crest of the ilium between the tensor fasciae latae and the vastus externus to the femur. They are abductors of the leg.

(3) The *ileo-fibularis* is the slender muscle next to the vastus externus on the dorsal side of the thigh. It extends from the ilium to the upper end of the tibio-fibula, draws the thigh dorsally, and flexes the shank.

(4) The *semimembranosus* is the posterior muscle of the dorsal side of the thigh

It arises from the ischial symphysis, is inserted on the tibio-fibula, bends the shank, and draws the leg toward the median line (*adduction*).

(5) The *gracilis major* and *minor* (also called *rectus internus major* and *minor*) are the posterior muscles of the ventral side of the thigh on the other side of the leg from the preceding. The minor is small and the more posterior one. Both extend from the ischium to the knee and have the same action as the preceding muscle.

(6) The *adductor magnus* is the muscle next anterior to the gracilis major on the ventral side. Most of it is concealed by gracilis major and the muscle to be mentioned next. It originates on the ischial and pubic symphyses and is inserted on the femur. It adducts the thigh and leg.

(7) The *sartorius* is the flat thin muscle crossing the lower end of the adductor magnus. It arises on the pubic symphysis and joins the general tendon over the knee Action, bends the shank and adducts the thigh.

(8) The *adductor longus* is a thin flat muscle under the sartorius but generally peeping out along the latter's anterior border. It originates on the ilium, joins and acts with the adductor magnus.

(9) The *semitendinosus* is a muscle of peculiar shape under the gracilis major, which should be removed to see it. It has two separate tendinous heads from the ischium and two separate bellies, uniting to one tendon fastened to the upper end of the tibio-fibula. It acts like the gracilis major.

h) *Muscles of the shank:*

(1) The *gastrocnemius* has been sufficiently described.

(2) The *peroneus* is the only other muscle on the dorsal aspect of the shank. It extends from the general tendon over the knee to the lower end of the tibio-fibula and the ankle. It extends and twists the foot and brings the shank up against the thigh, as in swimming and leaping movements.

(3) The *tibialis* muscles are the small muscles of the shank lying next to the bone. There are three of them. The *tibialis anticus longus* covers the anterior surface of the tibio-fibula; it arises by a slender tendon from the lower end of the femur and soon divides into two bellies, which are attached by slender tendons to the ankle bones. Action, bends the ankle. Beneath the lower part of this muscle is the small *tibialis anticus brevis,* originating on the middle of the tibio-fibula, and inserted on the ankle, which it also flexes. The *tibialis posticus* is a long slim muscle on the ventral aspect of the shank, between the gastrocnemius and the tibio-fibula, to which it is attached along its entire length. It is likewise inserted on the ankle, which it flexes and twists.

(4) The *extensor cruris* is a small muscle on the anterior aspect of the shank, lying next to the upper two-thirds of the tibio-fibula and in contact dorsally with the tibialis anticus longus. It originates on the femur and straightens the shank in the leaping and swimming movements.

XVI. PHYLUM CHORDATA: ANATOMY OF A BONY FISH

Among the vertebrates are found two general types of structure, that related to an aquatic habitat and that related to a terrestrial habitat. The frog despite its semi-aquatic habits has the construction of a land animal. It possesses limbs fitted for progression on land and circulatory and respiratory systems adapted to breathing air directly. The aquatic type of vertebrate is best represented by fish. They cannot progress on land, being adapted for locomotion in water only. Their respiratory and circulatory apparatus is constructed for utilizing the oxygen dissolved in the water.

In view of the fact that all vertebrates are anatomically similar in a number of respects and that the anatomy of the frog was considered in great detail, a thorough account of the anatomy of fishes will not be undertaken. Only the more important features will be considered.

Almost any of our common fishes will serve for study. Preserved material will be used for the most part. Living fishes in aquaria with plants should be on hand also.

1. General features.—In a preserved or living specimen note general form of the body. Is it adapted for progression through water? Is the animal bilaterally symmetrical? Does it have definite anterior and posterior ends? Is there a well-developed head? What differences do you note in color or otherwise between dorsal and ventral surfaces? Compare with the frog. Which are the more expanded, lateral surfaces or dorsal and ventral surfaces? Compare with the frog. Do you know of any fishes that are flattened dorso-ventrally like the frog?

The body is indistinctly divided into *head, trunk,* and *tail.* As in the frog there is no *neck.* The greater part of the body is clothed with an *exoskeleton* consisting of *scales* arranged in diagonal rows. Several *fins* are present.

Along each side of the body runs a curved line near and parallel to the mid-dorsal line. This is the *lateral line* and consists of a row of sense organs which serve to detect vibrations in the water.

The head is similar in some respects to that of the frog, considerably different in others in relation to the aquatic mode of life. As in the frog it terminates anteriorly in a large *mouth opening* bounded by *upper* and *lower jaws.* Open the jaws and note that not only is the lower jaw movable but some of the bones of the upper jaw are likewise movable so that the mouth can be widely opened and in some fishes protruded forward like a snout. This arrangement is of service in the capture of food.

The head bears the usual three pairs of sense organs characteristic of vertebrates. On the dorsal surface just back of the upper jaw are seen two small openings on each side, the *nostrils.* Although there are four nostrils, but two *nasal cavities* are present as in other vertebrates. Each of these has two openings to the exterior in the majority of fishes and the anterior one of the two is commonly provided with a *valve* or fold of skin which partly covers the opening. The arrangement of the nostrils permits a current of water to circulate through each nasal cavity, the water entering through one nostril and

passing out through the other. The large *eyes* are noticeable on the sides of the head. Are there any *eyelids?* The *ears* are situated behind the dorsal part of the eyes but are quite invisible externally. In fishes neither an external ear, such as we possess, nor a middle ear, such as we noted in the frog, is present but only that portion of our ears which is designated the *internal ear* exists. This is buried in the skull in the region indicated.

The posterior part of the head on each side is formed of a large flap consisting of flat thin bones overlaid by a thin layer of flesh. This flap is called the *gill cover* or *operculum*. Between the operculum and the body is a wide slit, the *gill opening*. Raise the operculum and look into the gill opening. Four arch-shaped structures, the *visceral arches,* or *gill arches,* are seen which bear on their external surfaces numerous filaments, the *gill filaments*. The set of filaments borne by one gill arch is called a *branchia* or *gill*. Attached to the ventral margin of each operculum is a membrane, the *branchiostegal membrane,* supported by bony rays, the *branchiostegal rays*. Spread the membrane out to see these rays. The structures described in this paragraph are used in respiration and the manner in which they operate will be explained later.

The principal features noticeable on the trunk are the *fins*. These are of two kinds, *median unpaired fins* and *lateral paired fins*. Of the former there are commonly present the following: one or two *dorsal* fins along the mid-dorsal line; the *caudal* or *tail* fin at the posterior end of the body; and a *ventral* or *anal* fin in the mid-ventral line shortly in front of the tail. The paired fins are two in number and correspond to the fore and hind limbs of land vertebrates. The fore or *pectoral* fins are situated one on each side shortly behind the operculum. The hind or *pelvic* fins are located on the ventral surface, close together, and usually not very far behind the pectoral fins. All of the fins are supported externally by parallel bony structures which are called *fin rays*. These are generally soft and flexible but in some of the median fins may be spinelike.

In a living fish note use of the various fins. How is the tail fin used? Does the trunk take part in vigorous swimming movements? Note which fins are employed when the animal remains stationary in the water.

In the median ventral line just in front of the anal fin is a large opening, the *anus* or *vent,* the end of the digestive tract Behind this is a depression in which projects a small elevation, the *urogenital papilla,* on which are borne the very small openings of the urinary and genital systems.

The fish possesses the same systems of organs which were described in connection with the frog (see p. 9) and which are possessed by complex animals in general. Each of these will now be studied.

2. **Integumentary system.**—This system includes the skin and its derivatives. The skin of the fish is microscopically similar to that of the frog, studied in the chapter on histology. It will be recalled that the skin is composed of two layers, the *epidermis* and the *dermis*. In the fish the epidermis contains numerous one-celled mucus glands which secrete on the surface the slime with which the animal is covered in life. The epidermis also contains the pigment cells which give to the animal its colors for the most part.

The fish unlike the frog is covered by a protective armor of scales which constitute

its *exoskeleton.* Note the arrangement of the scales, and differences in their size in different parts of the body. Do they occur on the head or operculum? Although apparently constituting the external surface of the body the scales are really formed by and set into the dermis of the skin, the epidermis being reduced to a thin film coating the free part of each scale. Grasp a scale with a forceps and remove it from the body. This act shows that only part of the scale is visible on the surface, the anterior half of it being set into a pocket in the dermis. Note scalloped margin of the edge that was attached. Examine a scale in a drop of water under low power of the microscope. Observe the concentric markings or *lines of growth* on the scale. From these the age of the fish can be determined as they are close together during the winter season, farther apart during the summer. Note the rows of minute *teeth* and the film of *epidermis* on the free part of the scale. The latter may contain black and colored *pigment cells.* Owing to the toothed border this kind of scale found on our common fishes is known as a *ctenoid* scale from a Greek root, *kten,* meaning comb. Ctenoid scales are composed of bone. **Draw a scale.**

The external fin rays, also called the *dermal fin rays,* are also formed by the dermis of the skin and hence belong to the exoskeleton. They are composed of bone. Examine the flexible fin rays with a hand lens. Note that they are composed of numerous small joints, and that they branch as they proceed toward the free border of the fin. Are the spiny fin rays jointed or branched?

3. **Muscular system.**—Make a cut through the skin at the base of the tail and strip off a piece of skin in the anterior direction. Note that under the skin the entire body is clothed in a thick layer of muscles. This layer is divided up into zigzag bands of muscle tissue. Each such band is called a *muscle segment* or *myotome.* Each myotome extends from mid-dorsal to mid-ventral line and is separated from the adjacent myotomes by a white partition consisting of connective tissue and called a *myocomma.* Observe that there is also a horizontal longitudinal partition corresponding in position to the lateral line and separating the myotomes into dorsal and ventral halves.

This arrangement of the musculature seen in the fish is the primitive vertebrate arrangement from which the muscular system of all land vertebrates is derived. It consists essentially of a longitudinal series of muscle segments. It is evidence of the primary segmental structure of the vertebrate body, there being as many segments in the body as there are myotomes. The myocommas may be regarded as corresponding to the rings seen on the surface of the body of the earthworm.

4. **Endoskeleton.**—The endoskeleton, as we have already learned, constitutes the internal framework and support of the body and is buried in the musculature. The reason for this is that the body musculature and the endoskeleton have the same embryonic origin. The endoskeleton of some fishes such as sharks is composed wholly of cartilage but in our common fishes this cartilage has been for the most part *ossified,* that is transformed into bone. In the chapter on histology we saw the microscopic structure of cartilage and bone. These should be recalled at this place. Owing to the fact that their skeletons are largely bony, our common fishes are known as *bony fishes.*

The endoskeleton of the fish consists of much the same parts that were seen in the study of the frog and that compose the human frame. It is divided into two general

parts, the *axial* skeleton and the *appendicular* skeleton. The former includes the *skull, vertebral column,* and supports of the unpaired fins; the latter the supports of the paired fins. A sternum or breastbone is lacking in fish.

Examine a prepared mounted skeleton of a bony fish. Identify the *skull,* the bony framework of the head; the *vertebral column,* the long structure composed of a series of bones, the *vertebrae,* each of which bears slender projecting processes; and the supports of the fins.

a) *Skull:* The skull is a complex structure composed of several parts of which the principal ones are: the *cranium,* the *sensory capsules,* and the *visceral skeleton.* These parts, originally separate, are now more or less united together to form the adult skull. The cranium is that part of the skull which incloses and protects the brain. It is, therefore, hollow, its cavity being known as the *cranial cavity.* The sensory capsules are skeletal parts which inclose or support the three pairs of sense organs of the head, namely, the nasal sacs, eyes, and internal ears; but the capsules of the eyes usually do not become ossified. The olfactory and auditory capsules, originally separate from the cranium, are in the adult incorporated with it as part of the skull. The visceral skeleton consists of a series of paired curved structures, the *gill arches,* which serve chiefly as supports of the gills. They are found in the ventral part of the head of the fish between the ventral parts of the two opercula. There are typically seven pairs of gill arches of which in the adult the first two pairs are considerably modified.

The skull of a bony fish is composed chiefly of bone but some cartilaginous portions persist unossified. These are not noticeable in dried specimens. In primitive fish, such as sharks, the entire skull is composed of cartilage. In bony fishes, the cartilage has undergone ossification to a greater or less degree in different fishes. Bones formed in this manner from the ossification of cartilage are known as *cartilage* bones. In addition to such bones there are in the skulls of bony fishes many bones which are not derived from cartilage but are formed directly by the dermis of the skin. Such bones are known as *dermal, membrane,* or *investing* bones. They have the same origin as the scales of the skin and may, in fact, be regarded as formed by the fusion of scales or teeth (teeth being modified scales). The flat thin bones one sees on the surface of the skull of a bony fish are membrane bones.

Study the skull. At its anterior end are situated the upper and lower jaws, both of which are movable. The most noticeable bones of the upper jaw are the two curved *premaxillae* which bound the mouth opening and the *maxillae* just behind and parallel to them. The principal parts of the lower jaw are the two *dentaries* bounding the mouth opening and the *articulars* behind the dentary, one on each side. Premaxillae, maxillae and dentaries are membrane bones and commonly bear teeth. On the dorsal surface near the anterior end of the skull are seen the nostrils which lead into the nasal capsules composed of several small bones and cartilages and inseparably incorporated into the skull. Posterior to the nasal capsules the roof of the skull is formed chiefly by two large flat membrane bones, the *frontals.* Posterior to them the roof of the skull terminates in a single median cartilage bone, the *supra-occipital,* generally provided with a median crest. To each side of the supra-occipital is a region containing the internal ear and composed of a number of *otic* bones. On each side of the skull is a large depres-

sion, the *orbit,* in which in life the eye is held. Surrounding this on ventral and posterior sides is a semicircular series of membrane bones, the *suborbitals.* The sides of the posterior part of the head are formed of the *opercular* bones, thin flat membrane bones, generally four in number on each side, which constitute the bony part of the operculum or gill cover. In front of the operculum is usually a series of three flat thin bones, which extend from the rear end of the lower jaw dorsally parallel to and somewhat overlapped by the suborbitals. These are named the *quadrate,* the *symplectic,* and the *hyomandibular,* beginning ventrally. They are cartilage bones. This series of bones supports the lower jaw and hence is termed the *suspensorium* of the jaw. The articular bone of the lower jaw articulates with the quadrate by a hinge joint. There are generally several cartilage bones known as *pterygoids* which extend forward from the suspensorium, pass to the inner side of the orbit, and are fastened to the under surface of the region of the nasal capsules. They are part of the upper jaw. Look into the mouth opening. Note teeth on the under surface of the nasal capsules, borne on the *vomer* bones. Another pair of tooth-bearing bones, the *palatines,* extend obliquely backward from the vomers. The middle of the roof of the mouth cavity is occupied by a single long bone, the *parasphenoid.* The palatines are cartilage bones, the parasphenoid and vomers, membrane bones.

The visceral skeleton lies ventral to the cranium and is composed entirely of cartilage bones. As already stated, it consists primitively of seven gill arches. Of these, the first, known as the *mandibular arch,* has been modified into the upper and lower jaws, already studied. The cartilage bones of these jaws, i.e., the palatines, quadrates, pterygoids, and articulars, represent the remains of the mandibular gill arch, and have, of course, no longer any gill-bearing function. Behind the lower jaw is seen the second gill arch, or *hyoid arch.* Each half of the hyoid arch extends dorsally to the inner side of the operculum which is fastened to it. The hyomandibular and symplectic bones are also parts of the hyoid arch. The hyoid arch bears the branchiostegal rays which are seen as curved bony rays along the ventral margins of the opercula. The two halves of the hyoid arch meet in the mid-ventral line to form a median unpaired projecting piece, the *basihyal,* which supports the tongue. It will be seen that the hyoid arch also has no gill-bearing function but supports the lower jaw, tongue, and opercula.

Behind and parallel to the hyoid arch are located the five typical *branchial* or *gill arches* of which the first four are gill-bearing. Each arch has the general form of the letter **U**, being composed of two curved halves whose dorsal ends are attached by tissue to the under surface of the cranium and whose ventral ends articulate with a series of bones in the mid-ventral line, called the *basibranchials.* The inner surface of each of the first four branchial arches commonly bears spinelike processes known as *gill rakers,* in one or two rows; these serve to strain out large particles. The outer surface of each of these arches bears numerous slender processes, the *gill rays,* which in life support the gill filaments. The last branchial arch is reduced in size and usually bears neither gill rakers nor gill rays. Teeth may also be present on the inner surface of the branchial arches; these differ in shape and arrangement in different fish.

b) *Vertebral column:* This consists of a longitudinal series of similar bones, the *vertebrae,* which form an axis for the trunk and tail. On the mounted skeleton note dif-

ferences between trunk and tail vertebrae. Isolated vertebrae should be available for study. Study the structure of an isolated tail vertebra. It consists of a central part, the *centrum,* drum-shaped, deeply concave at each end. From its dorsal surface spring two processes which soon unite forming an arch, the *neural arch,* which terminates dorsally in a long spine, the *neural spine.* Ventrally there is a similar *haemal arch* and *haemal spine.* In the center of the centrum is a small opening through which in life the *notochord* passes. The notochord is an elastic rod which in the embryo occupies the position and function of the vertebral column; the vertebral column forms around it and in fishes the notochord persists in the center of the centra of the vertebrae throughout life. **Draw** a tail vertebra.

Examine a trunk vertebra. Centrum, neural arch, and neural spine are similar to those of the tail vertebra but the haemal arch and spine are seen to be absent. Instead there is a lateral projection on each side of the centrum, called the *transverse process,* and to this is fastened a slender curved bone, the *rib.* The transverse processes may be regarded as formed by the opening out of the haemal arches. The steps in this process can be seen by studying the vertebrae at the junction of tail and trunk, on a complete mounted skeleton. Note differences in the length of ribs in different parts of the trunk. In some fishes each vertebra bears not only one but even two or three pairs of ribs, situated dorsal to the usual ribs and imbedded in the muscle layer. **Draw** a trunk vertebra with ribs.

On the entire mounted skeleton observe that a continuous canal is present dorsal to the centrum inclosed by the neural arches of the series of vertebrae. This canal is called the *neural canal* and in life contains the spinal cord. A similar canal, the *haemal canal,* is formed by the successive haemal arches in the tail; in life this contains blood vessels, hence the name haemal.

c) *Endoskeleton of the unpaired fins:* Observe the series of spinelike bones which support the unpaired fins (except the tail fin). They alternate with the neural and haemal spines of the vertebrae. Study the endoskeleton of the tail fin. Note that the tail fin is supported by the haemal arches of the last tail vertebrae. These arches are much broadened and flattened for this purpose. Observe further that the centrum of the last tail vertebra is much elongated and turns sharply dorsally bringing the expanded haemal arches into a suitable position for the support of the tail fin.

d) *Endoskeleton of the paired fins:* The pectoral fins are borne upon a **U**-shaped arch of bone, called the *pectoral girdle.* This consists of several bones. Its dorsal ends are fastened to the posterior end of the cranium. The pelvic fins are supported by a *pelvic girdle* which is not very typical in form, being degenerate or possibly primitive. It consists of two flat plates of bone, usually triangular in form, and generally more or less united in the mid-ventral line.

5. Respiratory system.—The greater part of this system was already seen. Cut off the operculum of one side and study the gill-bearing *visceral* arches. How many are there? Lift them up successively and note the wide slit or *gill slit* between successive arches. Where do the gill slits lead internally? Pass a probe into the mouth opening and see if it will come out freely through the gill slits.

Cut out a piece of one gill-bearing arch and study it in a dish of water. Note the

gill rakers: position? shape? number of rows? Study the gill filaments, the slender projections borne on the outer surface of the arch. Observe that they are double, that is, arranged in two parallel rows on each arch. This double row of filaments on each arch constitutes a *gill*. Note that the gill filaments are somewhat stiff, owing to the skeletal rod, the gill ray, inside each one. At the cut ends of the piece of arch note the bone of the bony arch inside, already seen in the study of the skeleton. **Draw** the piece of arch.

The respiratory system operates as follows. The mouth of the fish opens and simultaneously the opercula move outward and the branchiostegal membrane unfolds closing the gill opening. Owing to the outward movement of the opercula water is drawn into the mouth cavity. The mouth then closes, the branchiostegal membrane folds up again and by the inward movement of the opercula the water taken in is forced through the gill slits, thus bathing the gills, and out of the gill opening. The gill filaments contain many capillaries filled with blood which gives up carbon dioxide and acquires oxygen from the water through the thin walls of the gill filaments. The fish thus uses the oxygen dissolved in the water.

In a living fish note all of the foregoing movements. Observe opening and closing of mouth, operculum, and branchiostegal membrane, and time relations of these movements. Observe if the rate of these movements changes if the fish is disturbed and caused to move about rapidly.

6. Digestive system and coelome.—

a) *Mouth cavity and pharynx:* Open the mouth and place one blade of a scissors in the mouth cavity. Make a cut on the side where you removed the operculum, cutting through the angle of the jaws and across the middle of the visceral arches of that side, extending the cut through the last gill-bearing arch. Spread the walls of the cut apart and study the interior.

The large cavity revealed consists of an anterior part, the *mouth* or *buccal cavity*, and a posterior part, the *pharynx*, whose side walls are composed of the visceral arches.

In the roof of the mouth cavity study the distribution of *teeth*. They occur on the premaxillary bones, often on the maxillae also. There is also commonly a patch of vomerine teeth in the center of the anterior part of the roof and palatine teeth extending obliquely backward on each side from the vomerine teeth. Teeth may also be present in the roof of the pharynx borne on the dorsal parts of the gill arches. In the perch, for instance, there are two large patches of teeth in this location. Note also distribution of teeth in the lower jaw, on the dentary bones.

At the tip of both jaws just behind the premaxillary and dentary teeth, respectively, is situated a thin transverse membrane. These two membranes known as the *oral valves* prevent water from flowing out of the mouth in the respiratory movements.

In the floor of the mouth cavity note the *tongue*, a stiff pointed structure, which, unlike our own, has very little flexibility. Feel in the tongue its internal skeletal support, the basihyal bone, mentioned in the study of the gill arches. Note relation of tongue to tip of lower jaw and to the visceral arches.

The principal feature noticeable in the pharynx are the six visceral arches. Note that these form the walls of the pharynx. The slits between them are the gill slits. The

first (really second, as the jaws constitute the first) is the hyoid arch. Note how this extends obliquely backward on each side from the tongue to the operculum, leaving a deep groove between itself and the margins of the lower jaw. Behind the hyoid are the remaining five arches. Note again on them the gill rakers and filaments, and feel with the fingers the bony arch inside of each one. Observe that the last arch is reduced and does not bear any rakers or gill.

We may note that three structures prominent in the mouth cavities and pharynx of land vertebrates and seen in the study of the frog are completely absent in the fish. These are: the internal nares, the openings of the auditory tubes, and the glottis. The nasal cavities of the fish do not open into the mouth cavity, for the fish does not use its nasal cavities in respiration. The fish has no middle ears and hence the auditory tubes which in land vertebrates lead from the pharyngeal cavity to the middle ears are not present. The fish has no lungs and hence no glottis. On the other hand we find in the walls of the pharyngeal cavity structures, the visceral arches and gill slits, which are lacking in the adults of land vertebrates though present in their embryos. As we learned, the frog tadpole possesses these structures in a condition identical with that of the fish. In the adults of land vertebrates the skeletal portions of the gill-bearing visceral arches persist in part as the cartilages of the larynx, and hyoid apparatus.

Make a drawing of the opened mouth and pharyngeal cavities showing all structures in them.

b) *The coelome and its contents:* Make an incision in the mid-ventral line from a point just in front of the anus to the branchiostegal membranes, cutting through the pectoral girdle. Spread the cut apart making transverse cuts in each wall if necessary. A large cavity is revealed which is the principal part of the *coelome* or *body cavity*. As in the frog, this cavity is named the *pleuroperitoneal cavity* (often but erroneously designated the abdominal cavity). It is lined by a smooth shining membrane, the *peritoneum,* and contains a number of *viscera*. The more conspicuous of these may be noted now but will be examined in more detail later. The coils of the *digestive tract* usually imbedded in folds of fat are the most noticeable objects. At the anterior end of the cavity is the *liver*. The stout body or pair of bodies in the posterior part of the cavity dorsal to the digestive tract are the *gonads*. Observe that the viscera are supported by *mesenteries* as in the frog.

The anterior end of the pleuroperitoneal cavity is closed by a transverse partition, the *transverse septum* (also called the *false diaphragm*). This is situated just anterior to the liver which is attached to it. Carefully and slowly deepen your cut in the mid-ventral line in the region anterior to the transverse septum. The cut will soon strike a cavity which lies immediately in front of the septum and between the visceral arches of the two sides. This cavity is the *pericardial cavity*. Open the pericardial cavity and observe that it contains the *heart*. It is a cavity of conical form lined by a smooth membrane, the *pericardium,* which about corresponds to the pericardial sac of the frog. The posterior part of the pericardium forms the anterior face of the transverse septum; the posterior face of the septum is formed of peritoneum. The pericardial cavity is a portion of the coelome. The coelome of the fish is thus seen to consist of two cavities, the pericardial and pleuroperitoneal cavities separated by the transverse septum.

Note that the body wall as in the frog is composed of three layers—skin, muscles, and peritoneum.

c) *Parts of the digestive tract:* Remove the left wall of the pleuroperitoneal cavity. Study the shape of the liver. Note its attachment to the posterior face of the transverse septum by the *coronary ligament*. On the left side dorsal to the liver note the *esophagus*, a stout white tube emerging from the posterior end of the pharynx. The esophagus passes insensibly into the *stomach*. The stomach may be of simple **U**-form, but in some fishes as the perch the greater part of the stomach consists of a saclike posterior projection. Are esophagus and stomach attached to the mid-dorsal line by a mesentery? From the end of the stomach or from its middle in the case of the perch extends a narrow tube which is constricted immediately upon leaving the stomach. This constriction is the *pylorus* and the remainder of the digestive tract beyond the pylorus is the *intestine*. This cannot be very definitely divided into small and large intestine but it is probable that the greater part of it corresponds to the small intestine of other vertebrates. The part of the intestine immediately beyond the pylorus is named as usual the *duodenum* and commonly is encircled by finger-like projections, the *pyloric caeca*, which vary in number from one to two hundred in various species of fish. In the perch there are three of these caeca. They are believed to secrete digestive enzymes. Beyond the pyloric caeca the duodenum curves anteriorly toward the liver. Examine the posterior surface of the liver at this place and locate the *gall bladder*. If possible find the *bile duct* leading from the gall bladder to the duodenum. The intestine now proceeds posteriorly, then makes a loop anteriorly, and then extends straight to the anus. All of these parts are more or less buried in fat which should be stripped off. Between the duodenum and the anterior loop of the intestine is a small dark body, the *spleen*. The *pancreas* is so diffuse that it cannot be located definitely by dissection. Note mesenteries between parts of the digestive tract.

Make a drawing of the digestive tract.

7. The reproductive system.—This consists of a pair of *gonads* which may in some fishes in one or both sexes appear single owing to their fusion. The single or paired testis is commonly white while the single or paired ovary is generally colored and further recognizable by the numerous eggs of which it is composed. Note mesenteries supporting the gonads. The size of the gonads varies greatly according to the season. When fish are sexually mature the gonads commonly fill the greater part of the pleuroperitoneal cavity. There are no ducts leading from the gonads in the great majority of bony fishes; nor is there any connection between the male gonads and the kidneys as is the case in the males of most other vertebrates. Each gonad simply tapers to the urogenital opening through which the eggs or sperm as the case may be are discharged in most bony fishes. Some fish, however, are viviparous.

In some of the bony fishes the two sexes may be distinguished externally by differences in color, size, or shape. The fins of the male, particularly the anal fin, may be strikingly different from those of the female. In most fishes, however, dissection alone reveals the sex of the animal with certainty.

8. The swim-bladder or air-bladder.—The entire dorsal wall of the pleuroperitoneal cavity of most bony fishes is occupied by a large gas-filled sac, the *swim-bladder*

or *air-bladder.* Its presence is readily noted on pressing the region indicated. The peritoneum passes over the ventral face of the swim-bladder. The swim-bladder is connected with the pharynx in some fishes but not in others (not in the perch). With a fine scissors slit open the swim-bladder along one side and note its size and shape. In some fishes (not the perch) the interior is divided up by partitions. The swim-bladder in life contains gases which are not necessarily in the same proportion as in the air. By changing the amount or kind of gases in the bladder the fish may change its volume and thus adjust itself to different depths of water. By increasing its volume the fish rises in the water and by decreasing it is able to descend to greater depths.

9. Excretory system.—This consists of a pair of *kidneys* and their *ducts.* Each kidney is a long narrow dark colored irregular strand lying in the roof of the swim-bladder, one just to each side of the mid-dorsal line. Trace them forward and note that at the anterior end of the pleuroperitoneal cavity, dorsal to the esophagus, the two kidneys unite into a soft reddish mass, frequently but erroneously called the *head-kidney.* Investigation of this mass shows that it is not composed of kidney tissue at all but is a *lymph gland,* similar to the spleen, that is an organ which makes new blood corpuscles and destroys old worn-out ones. Each kidney has a duct, the *mesonephric duct,* which runs the entire length of the kidney. The mesonephric ducts are best seen at the posterior ends of the kidneys. Carry the mid-ventral cut backward to one side of the anus. Clean away tissue at the posterior ends of the kidneys and note the two mesonephric ducts which proceed from these ends and pass along the posterior wall of the pleuroperitoneal cavity ventrally to the urogenital papilla located in a depression behind the anus. Before reaching this the two ducts unite and enter an expanded portion, the *urinary bladder,* situated just dorsal to the posterior termination of the gonads.

Draw excretory system.

10. Circulatory system.—The circulatory system, particularly the venous system, is unsatisfactory to study in preserved specimens. Freshly killed specimens should be used if possible. The following description applies chiefly to the perch.

As stated in the study of the frog, vertebrates have two circulatory systems, the *lymphatic* and the *blood-vascular* system. The latter is usually meant when the expression circulatory system is employed and only this system will be studied in the fish. It consists as in all vertebrates of a *heart,* a *venous system* (system of blood vessels in which the blood flows toward the heart), an *arterial system* (blood vessels in which the blood flows away from the heart), and the *capillaries,* microscopic vessels connecting arteries and veins.

a) The chambers of the heart: The heart has already been noted as situated in the pericardial cavity located just anterior to the transverse septum. Open the pericardial cavity as before if a new specimen is provided. The heart of bony fishes consists of but three chambers: the *sinus venosus,* the *auricle,* and the *ventricle.* The *ventricle* is the pale, thick-walled, cone-shaped structure readily noticed in ventral view of the heart. At the sides of and dorsal to the ventricle is the larger, thin-walled, dark colored *auricle.* Posterior to the auricle and ventricle is the *sinus venosus,* a transversely elongated chamber imbedded in the transverse septum. The sinus is readily seen if the ventricle is raised and stretched forward.

b) *The ventral aorta and the afferent branchial arteries:* From the ventricle a large vessel, the *ventral aorta*, proceeds directly forward in the median line. It is greatly enlarged just in front of the ventricle, forming the *bulbus arteriosus,* erroneously stated in many books to be a part of the heart. The bulbus arteriosus is simply a muscular enlargement of the beginning of the ventral aorta serving the purpose of increasing the pressure on the blood. A conus arteriosus is lacking in the majority of bony fish.

Trace the ventral aorta forward carefully slitting away muscles, etc., to reveal its course. It passes forward between the ventral ends of the visceral arches into which it sends off branches, called the *afferent branchial arteries.* These are best seen after injecting the ventral aorta. This can readily be done as follows. Draw out a medicine dropper to a fine tube. Fill this with a thin colored solution (a brightly colored ink does very well). Make a small slit in the bulbus arteriosus, insert the end of the dropper into the slit, push it forward into the bulbus, and by gentle pressure fill the afferent branchial arteries with the colored fluid. There are three pairs of these arteries, arising at equal distances from the anterior part of the ventral aorta. The third pair forks in two immediately on leaving the aorta, so that there are four pairs of the afferent branchial arteries. Each proceeds at once into the ventral end of the adjacent visceral arch. Examine the arches and note the afferent artery running along each arch just external to the bony gill arch. From each afferent branchial artery fine vessels extend into the gill filaments.

Make a drawing from ventral view of the heart, ventral aorta, and afferent branchial arteries.

The heart of fishes contains only venous blood which is sent out by means of the afferent branchial arteries into the gill filaments where it is aerated.

c) *The systemic veins: Systemic* veins are those which empty directly into the heart. In a fish all of the systemic veins open into the sinus venosus. Turn the ventricle forward and examine the sinus venosus. It is continuous at each side with a large vein, the *common cardinal vein,* lying in the transverse septum, and appearing as lateral prolongations of the sinus. The sinus venosus receives in the middle part of its posterior wall two *hepatic* veins from the liver. These are readily seen by stretching the liver backward and looking in the coronary ligament; note the two veins passing from the liver into the transverse septum. All of the other veins open into the common cardinals which as noted are continuous with the sinus. On the right side a vein of fair size proceeds along the roof of the pericardial cavity and empties into the right common cardinal. This is the right *inferior jugular vein.* The left one is small or absent. Trace this vein forward in the median line just dorsal to the ventral aorta. At the lateral border of the transverse septum, each common cardinal vein is seen to be formed by the union of two veins, the *anterior* and *posterior cardinals.* Each anterior cardinal turns dorsally, running along the posterior wall of the gill opening, and then anteriorly above the dorsal margin of the operculum. It is best followed by making a slit in the common cardinal vein, inserting a probe in the slit, and following the course of the anterior cardinal vein with the probe. The anterior cardinal veins of fishes are the same as the precaval veins of other vertebrates. The posterior cardinal veins are large vessels which curve

posteriorly. Follow them by dissecting away the lateral walls of the transverse septum. The posterior cardinal veins pass through the lymphatic part of the kidneys mentioned above, then run posteriorly along the kidneys, one to each side of the mid-dorsal line. The right posterior cardinal runs the entire length of the kidney and continues into the tail as the *caudal vein,* located in the haemal canal of the vertebrae. The left posterior cardinal is shorter and terminates in the left kidney. *Renal* veins from the kidneys and *parietal* veins from the body wall enter the posterior cardinals at intervals. The latter may also receive veins from the swim-bladder.

Draw the systemic veins as far as seen.

d) *The portal systems:* A *portal system* is a system of veins which do not flow directly into the heart but break up into capillaries in some organ. In fishes as in the frog there are two portal systems, the *hepatic portal* in which the interposed capillaries are in the liver, and the *renal portal* where they are in the kidney. The hepatic portal vein is a large vein which enters the liver from behind. Turn the liver forward and look for it near the bile duct in the mesentery connecting liver and duodenum. The hepatic portal vein receives branches from the intestine, stomach, spleen, and swim-bladder. Some of these may be seen, depending on the state of the specimen. The vein can be injected backward as directed above for the ventral aorta in order to render its tributaries visible. The hepatic portal vein breaks up into capillaries in the liver, the blood being collected again by the hepatic veins. The student should understand from texts the purpose of this arrangement whereby the blood from the digestive tract must pass through the capillaries of the liver.

The renal portal system is not practical to find and varies in different fish. In the perch it consists of a branch from the caudal vein which breaks up into capillaries in the left kidney from which the blood is again collected by the posterior cardinals. The purpose of the renal portal system is not very clear.

e) *The dorsal aorta and the efferent branchial arteries:* The principal artery of the fish is the *dorsal aorta* which will be found in the mid-dorsal line of the roof of the swim-bladder between the two posterior cardinal veins. Posteriorly it continues into the tail as the *caudal* artery lying in the haemal canal just above the caudal vein. These vessels can be seen by cutting across the tail behind the anus. As the dorsal aorta passes along the mid-dorsal line of the roof of the swim-bladder it gives off *renal* arteries to the kidneys and *parietal* arteries to the body wall. The main branches of the dorsal aorta arise at its anterior end. It is generally best to inject forward into the aorta before trying to study these branches.

The mouth and pharyngeal cavities were opened in an earlier stage of the dissection. If a new specimen is provided, insert one blade of the scissors in one angle of the jaw and cut across operculum and visceral arches of one side. Open the mouth and pharyngeal cavities as widely as possible. Cut through the lining membrane of the roof of the pharyngeal cavities midway between the dorsal ends of the visceral arches, in the perch midway between the two large teeth in the roof of the pharyngeal cavity. With a probe peel away the lining membrane to each side of the cut and carefully dissect in the tissue dorsal to the lining until you disclose the *efferent branchial arteries* coming from the dorsal ends of the visceral arches. There are four pairs of these. On

each side the first and second join separately a curved vessel, sometimes called the *suprabranchial* artery. Beyond the entrance into them of the second efferent branchials, the two suprabranchial arteries curve toward the mid-dorsal line where they meet to form the dorsal aorta. At the union, the third and fourth efferent branchials join the dorsal aorta. Follow the efferent branchials out into the visceral arches. Note in each visceral arch an efferent branchial artery lying just external to the afferent branchial previously seen. Dissect along the first efferent branchial and at the place where it joins the suprabranchial note that the latter extends forward into the head as the *carotid* artery.

Cut away the attachment of the esophagus to one body wall and follow the dorsal aorta posteriorly. Immediately beyond the entrance into it of the third and fourth branchials a large *coeliac* artery arises from the ventral surface of the aorta and branches extensively to the digestive tract, gonad, and swim-bladder. Just beyond the origin of the coaliac artery the paired *subclavian* arteries spring from the dorsal aorta and supply the pectoral fins.

Make a drawing of the efferent branchial arteries and dorsal aorta and its branches.

The afferent and efferent branchial arteries together constitute vessels called the *aortic arches* because they are conceived as running in a curve through the visceral arches. Originally six in number in vertebrates but four are left in most fishes, the first two having disappeared. The present first, second, and fourth pairs correspond to the carotid, systemic, and pulmo-cutaneous arches of the frog.

11. The sense organs.—The principal sense organs of the fish are the olfactory sacs, eyes, internal ears, and lateral line system.

a) *The lateral line system:* This consists of the lateral line, already noted, and of similar canals, not very distinct, branching on the head. The lateral line is really a long canal with sense organs in its wall and opening at intervals to the exterior by means of minute canals and pores which pierce the scales covering the lateral line canal. Remove a scale from the lateral line and examine under the microscope. Note canal and pore in it. This system is allied to the ear and is used to perceive vibrations in the water.

b) *Olfactory sacs:* Cut away the skin over one olfactory sac and note the folds which occupy the interior serving to increase the amount of surface bearing the sensory cells of smell. Note lack of connection of the nasal with the mouth cavities.

c) *Eyes:* The exposed part of the eye is called its *front.* Note the transparent membrane forming the front of the eye, and internal to it a dark-colored membrane, the *iris,* with a central hole, the *pupil,* occupied by a pearly round body, the *crystalline lens.* There are no eyelids but a fold of skin surrounds the front of the eye.

Remove the roof of the skull between the two eyes. Proceed carefully as the brain lies beneath imbedded in fatty material. The brain must not be injured. The two white cords between the eyes dorsally are the *olfactory nerves.* Cut away the fold of skin around one eye noting as you do so that this fold is attached to the eye. Grasp the fold and pull on it firmly. It should pull away from the front of the eye as a thin transparent membrane, the *conjunctiva.* The removal of the conjunctiva reveals the *cornea,* a transparent coat which forms the front of the eye.

After cutting away tissue on all sides of the eye, the latter is revealed as a large rounded body, more properly termed the *eyeball* or *bulb* of the eye. It reposes in the *orbit,* a depression in the skull, to which it is attached by six *muscle bands* and the *optic nerve.* Study the muscle bands, cleaning away tissue from them. From the middle of the dorsal surface of the eyeball, two muscles extend, one, the *superior oblique,* forward to the anterior wall of the orbit, the other, the *superior rectus,* backward to the posterior wall. Raise the eyeball up and look on its ventral surface for two similarly placed muscles, an anterior one, the *inferior oblique,* and a posterior one, the *inferior rectus.* Look on the posterior surface of the eyeball for the *external rectus.* The *internal rectus* is just beneath the superior rectus and will be seen by cutting the latter at its attachment to the eyeball. By means of these six muscles the eyeballs of vertebrates can be turned in various directions.

Cut through all of the eye muscles at their attachments to the eyeball. Pull the eyeball outward and note that it is still attached to the orbit by a strong white stalk, the *optic nerve.* Cut through the nerve at its attachment to the eyeball and remove the latter. In the orbit note the origins of the six eye muscles, the two obliques from the anterior wall, the four recti from the posterior wall. Observe the optic nerve in the center of the four recti.

Study the structure of the eyeball. Cut it vertically into two unequal parts, leaving the lens in one part. Examine in a small dish under water. The eyeball is composed of three coats: an outer white tough coat, the *sclera,* which becomes the cornea in front; a middle *chorioid* coat, black on the inner surface, silvery on the outer surface, and becoming the iris in front; and the *retina,* a soft greenish layer lining the cavity. The retina is the nervous part of the eye containing the sensory cells of sight; it is continuous with the optic nerve. Note the round lens behind the pupil, held in place by the iris. The cavity between the cornea and the iris is called the *anterior chamber* of the eye, and in life is filled with a fluid, the *aquaeous humour.* The larger cavity between the iris and the retina is called the *vitreous chamber* and in life is filled with a gelatinous material, the *vitreous body.* The lens is attached to the retina near the beginning of the optic nerve by a strand called the *falciform ligament* which passes through the vitreous body.

Draw the section through the eyeball.

d) *Internal ears:* Remove the roof of the skull posterior to the eyes, noting beneath it fatty material in which the brain is imbedded. Remove also the dorsal mass of muscles behind the head. Note just behind the eye on each side a rounded elevation of the skull situated at the sides of the brain. These rounded elevations contain the internal ears. From textbooks learn the structure of the internal ear. It consists principally of three curved canals, the *semicircular ducts,* attached to a chamber, the *utriculus,* ventral to which is another chamber, the *sacculus.* Carefully shave off the bone in the elevations mentioned and try to find some of these structures.

12. The nervous system.—This is composed in vertebrates of the *brain, spinal cord, sympathetic system,* and *nerves* to and from these parts. The brain is inclosed in the cranial cavity and has already been exposed in part. Expose its dorsal aspect fully now by carefully picking out the fatty material surrounding the brain and cutting away

the sides of the skull. The brain is a white nervous mass expanded into a number of lobes. Its parts are the same as in the frog and other vertebrates.

a) *Dorsal aspect of the brain:* At the anterior end of the brain are the two very small *olfactory lobes,* from which the long *olfactory nerves* extend to the olfactory sacs, passing between the two eyes. Behind the olfactory lobes are two much larger lobes, the *cerebral hemispheres,* and behind these the still larger *optic lobes.* Posterior to the optic lobes is an unpaired median swelling, the *cerebellum.* Below and behind this is the *medulla oblongata* which narrows into the *spinal cord* at the rear end of the skull. The spinal cord continues down the neural canal formed by the neural arches of the vertebrae. Between the optic lobes and the cerebral hemispheres is a depressed area, which represents all that is visible dorsally of a part of the brain named the *diencephalon.* From it springs a slender process, the *pineal body* or *epiphysis,* generally torn off in the dissection.

Draw the dorsal aspect of the brain.

b) *The cranial nerves:* There are ten nerves springing from the brain but some of them are generally difficult to see in a small fish such as the perch.

(1) *First or olfactory nerve:* These are the long nerves already noted extending from the olfactory sacs to the olfactory lobes of the brain. They are the nerves of smell.

(2) *Second or optic nerves:* These were already seen in the orbit. Cut across the olfactory nerves, gently lift the anterior end of the brain, and note the two large optic nerves entering the ventral surface of the diencephalon. The optic nerves are not really nerves but parts of the brain. They convey the sight impulses.

(3) *Eye muscle nerves:* There are three pairs of cranial nerves for the six pairs of eye muscles. The third or *oculomotor* nerve supplies the inferior oblique and the superior, inferior, and internal recti. It arises from the brain just below the optic lobe on each side and extends forward into the orbit. The fourth or *trochlear* nerve supplies the superior oblique muscle and springs from the side of the brain between the optic lobe and the cerebellum. The sixth or *abducens* nerve, to the external rectus muscle, springs from the ventral surface of the medulla and cannot be seen at this time.

(4) *The fifth or trigeminus and the seventh or facial nerves:* These two nerves arise together and are readily seen as the large trunk springing from the side of the anterior end of the medulla. They pass obliquely forward in front of the internal ear. The trigeminus supplies sensory fibers to the upper and lower jaw and orbit and motor fibers to the muscles of the lower jaw. The facial supplies the lateral line organs of the head and is a motor nerve to muscles of the hyoid and mandibular arches, and the operculum.

(5) *The eighth or auditory nerve:* This nerve springs from the side of the medulla just behind the preceding and branches at once to the internal ear.

(6) *The ninth or glossopharyngeal and tenth or vagus nerves:* These arise together from the medulla by a large root just behind the origin of the eighth nerve. This passes obliquely backward along the posterior border of the internal ear to a point at the dorsal ends of the gill-bearing visceral arches, to which it sends nerves. The glossopharyngeal supplies the first gill-bearing arch, the vagus the others, and then proceeds posteriorly to supply various viscera and the lateral line.

c) *Ventral aspect of the brain:* Remove the brain from the skull by cutting across the cranial nerves and the rear end of the medulla and examine the ventral surface. The principal structure to be noted in ventral view is the *diencephalon* which now appears as a large mass to the ventral side of the cerebral hemispheres and optic lobes. At the anterior end of the diencephalon note the large optic nerves, which cross forming the *optic chiasma* before they reach the brain. They enter the sides of the diencephalon and the optic lobes. Back of the optic chiasma is a median swelling in the floor of the diencephalon called the *infundibulum*. To this is attached a rounded body, the *hypophysis* or *pituitary body*, generally torn off and left in the skull. Look for it in the floor of the skull situated in a cavity. Behind this the infundibulum bears a pair of swellings, the *inferior lobes*. Between the inferior lobes and the optic lobes are the roots of the oculomotor nerves and just behind them the roots of the trochlear. Look on the ventral surface of the anterior part of the medulla just to either side of the midventral line for the small roots of the abducens nerves. Identify other parts of the brain and roots of the other cranial nerves from ventral view.

Draw the brain from ventral view showing also the roots of the cranial nerves so far as found.

The cavities or *ventricles* of the brain are the same as in the frog and for them pages 135–36 may be consulted. The dissection of the spinal nerves and the sympathetic system is not very practical in a small fish.

XVII. FINAL EXERCISES ON COMPARATIVE ANATOMY

A. COMPARISON OF CROSS-SECTIONS

Make diagrammatic cross-sections through Hydra, Planaria, earthworm, and frog, putting in all the layers of the body. Color ectoderm blue, mesoderm red, entoderm yellow, peritoneum and mesenteries green. Indicate epithelial layers by crosslines at right angles to the surface; connective tissue by solid shading, muscles by diagonal lines, skeleton by dots. After finishing these diagrams study them and be sure that you understand thoroughly what layers correspond in the four animals, and in what important ways the sections differ from each other.

B. COMPARISON OF FUNCTIONAL SYSTEMS

Make a table as follows. Rule off a page with vertical and horizontal lines so as to make a number of vertical and horizontal columns. At the left, in the spaces between the horizontal lines, write the names of all the animals studied in this course, beginning with the Amoeba and ending with the frog, in their proper phylogenetic order. At the tops of the vertical columns, going from left to right, write the names of the systems in the following order: digestive system, muscular system, nervous system, reproductive system, excretory system, circulatory system, respiratory system, skeletal system. In the appropriate spaces opposite the names of the animals, write in very briefly whether the animal has such a system and if so what it consists of. The table when finished will illustrate the gradual increase in numbers of systems and complexity of each system through the animal kingdom. Note that a system consists of parts set aside for the performance of specific functions. Thus the Amoeba respires but it has no respiratory system, that is, no structures for that purpose.

XVIII. EXERCISE ON CLASSIFICATION

The science of *classification* or *taxonomy* is that branch of biology which attempts to discover the natural relationships of organisms, and to put all known forms of life in their proper places in a genealogical tree. Classification is based entirely upon anatomy, both adult and embryonic, and is not in the least concerned with function.

Classification starts with the conception of the *species*, which may be defined for our purpose as a group of organisms essentially alike in all the details of their structure. Thus all of the frogs given out in the laboratory are so nearly alike that the laboratory instructions even down to the microscopic structure apply to all individuals. Such frogs therefore constitute a species and are given a name, called the *specific name*, which is in the case of the leopard frog *pipiens*. There are many other kinds of frogs which are quite similar to this one but differ in small details; for instance, the bull frog is larger, has a different color pattern, and lacks the dermal plicae; it therefore receives another specific name, *catesbiana*. There are, in fact, about one hundred and forty different kinds of frogs, each of which has a specific name. In order to express the fact that all of these frogs are very similar to each other they are placed together into one group, called a *genus*. This genus to which the frogs belong is called *Rana*, from the Latin word meaning "frog," and this name is spoken of as the *generic* name. The full name of our common leopard frog is therefore *Rana pipiens*. This system of naming animals with two names is called the *binomial system* of nomenclature and was devised by Linnaeus, one of the early biologists who became interested in classification.

Among the members of a species there are often minor variations, which are distinguished as *varieties*, when sufficiently important and common. Varieties occur most frequently in domestic animals. Everyone is familiar with varieties or breeds of cattle, horses, chickens, dogs, etc.

All of those genera which are naturally related to each other as shown by their structure are united together into a *family*. Thus the lions, tigers, leopards, lynxes, and other catlike animals form a natural family, the *Felidae*, or cats, having sharp fangs, and retractile claws. Similarly the dogs, wolves, foxes, etc., form another natural family, the *Canidae*, in which the claws are not retractile; the bears are another family; so also the hyenas, and the otters, weasels, and martens. All of these families, together with many others which have not been mentioned, have certain characters in common, such as that they all are carnivorous and have strong fangs and sharp cutting teeth, that they have claws, and walk upon their tiptoes, so to speak, and that their skeletons are very similar. In recognition of these facts, which demonstrate that they are related to each other, they are united into a larger group, called an *order*, in this case, the order *Carnivora*. If we consider other animals familiar to us we find that they too fall into natural orders; thus the cattle, sheep, deer, horses, resemble each other in that they walk on their toenails, which are broadened into hoofs, their legs are much modified for running, and their teeth are broad and ridged for grinding up vegetable food. They

constitute another order, the *Ungulata*. Similarly the rabbits, squirrels, mice, rats, beavers constitute the order *Rodentia*, distinguished by sharp, chisel-like front teeth.

If we further consider these and other orders of familiar quadruped animals we find that they have certain large characters in common, such as that they are all clothed with fur, that their young are born alive and nourished with milk, and that their skeletons are quite similar. These facts point to undoubted relationships between them, so that they are united into a still larger taxonomic group, the *class*, in this case the class *Mammalia*, or the *mammals*.

The birds are another natural class, distinguished by their covering of feathers, modification of the fore limbs into wings, and egg-laying habit. Frogs, toads, and salamanders are another, with slimy smooth skins; snakes, lizards, alligators and similar animals form another class, with dry scaly skins; fishes constitute a class distinguished by the presence of gills and fins.

All of these apparently diverse classes of animals have further certain common characters, such as the presence of an internal cartilaginous or bony skeleton, consisting of skull, vertebral column, limb girdles, etc., two pairs of appendages, a ventral chambered heart, a dorsal nervous system, etc. They therefore together constitute one of the great divisions of the animal kingdom, a *phylum*.

The taxonomic divisions are therefore variety, species, genus, family, order, class, phylum. There are usually other subdivisions, also, as subphylum, subclass, suborder, superfamily, subfamily, etc. Naturally, the details of classification are not yet agreed upon because we know as yet little about the natural relationships of animals and because it is difficult to decide whether certain characters are as important as others, as, for example, whether certain differences between two animals will place them in different genera only, or whether they are great enough to separate them into two different families. For this reason the student need not be surprised to find that the various textbooks do not agree on the details of classification, although all recognize the same large groups.

There follows a key to the phyla and classes of the animal kingdom. Peculiar, rare, and aberrant forms are not provided for in the key, but an attempt has been made to include all animals commonly met with. With the aid of the key, classify ten different animals provided. In this key each statement of characters bears a number followed in parentheses by one or more numbers which refer to the alternative statement. If, therefore, upon reading the first statement the student decides that this does not fit the animal in question, he turns at once to the number or numbers in parentheses and continues to do this until he finds a statement that does fit. In this case he proceeds to the number given at the end of the statement. This method of making a key is taken from Ward and Whipple's *Fresh-Water Biology*. The key is in part derived from one devised by Dr. V. E. Shelford, of the University of Illinois. Only the simplest possible characters have been chosen as means of identification, even though this often involves repetition and increased length of the key.

The arrangement of the phyla in the following key is purely artificial and should not be regarded by the student as representing the evolutionary order.

Key to the Phyla of Animals

1 (2). Animals consisting of a single cell, or of a colony of like cells, or masses of multinucleate protoplasm; mostly microscopic. **Phylum Protozoa**

2 (1). Animals consisting of many cells, of several or many different kinds, arranged in definite layers. 3

3 (4, 11). Without definite symmetry. Forming sessile motionless crusts or masses, often branching; body porous, rough, and bristly, pierced by numerous holes, of which one or more are large and conspicuous. **Phylum Porifera**

4 (3, 11). With definite radial symmetry. 5

5 (10). Relatively simple animals, without anus, coelome, or definite organs. 6

6 (7). Sessile, vase-shaped, or cylindrical forms, porous, rough, bristly, with one large terminal, non-closeable opening; without tentacles. A few members of the **Phylum Porifera**

7 (6). Body not pierced by visible pores; nearly always with tentacles; may be naked or may be provided with external skeletons of horny or calcareous material; apical opening a closable mouth; parts of the body in fours or sixes or indefinite. 8

8 (9). With eight radial rows of ciliated swimming plates; tentacles, if present, without nematocysts. **Phylum Ctenophora**

9 (8). Without such rows of ciliated plates; with tentacles armed with nematocysts; often sessile and colonial, some free-swimming. **Phylum Coelenterata**

10 (5). More complex animals, with anus, coelome, and definite organs; parts of the body almost always in fives, sometimes indefinite; hard, spiny, or leathery animals; tentacles, if present, branched, and never with nematocysts. **Phylum Echinodermata**

11 (4, 3). With definite bilateral symmetry, at least in part of the body; sometimes spirally coiled in part; sometimes posterior end bent anteriorly toward mouth. 12

12 (34, 37). Without an internal cartilaginous or bony skeleton in the form of skull or vertebral column, wall of the pharynx not pierced with gill slits. 13

13 (14). With one or more girdles or crowns of cilia borne upon the discoidal, platelike, or lobed anterior end; with internal movable chitinous jaws; small or microscopic aquatic forms. **Phylum Trochelminthes**

14 (13). Without such crowns of cilia. 15

15 (27). Body not divided into segments, nor with segmentally arranged bristles, nor with segmented appendages. 16

16 (17, 20). Wormlike animals without an anus; flattened dorsoventrally, often leaflike or ribbon-like; free-living in water, or parasitic. **Phylum Platyhelminthes**

17 (16, 20). Wormlike animals with an anus; without an exoskeleton; heads poorly developed and not distinct from body. 18

18 (19). With a long proboscis inclosed in a sheath lying dorsal to the alimentary canal, capable of protrusion from the anterior end; slender, often very long, flattened to cylindrical worms, mostly marine. **Phylum Nemertinea**

19 (18). Without such a proboscis; slender cylindrical worms, pointed at each end, covered by a smooth thick cuticle; water, damp earth, or parasitic. **Phylum Nemathelminthes**

20 (17, 16). Generally not wormlike, with an anus; often provided with an exoskeleton, consisting of calcareous shells of one or more pieces, or calcareous or horny cases or tubes, or a gelatinous secretion; if naked, with much more definite heads than under 16 and 17. 21

21 (22). Colonial animals, sessile, fastened in cases, tubes, or on the surface of gelatinous masses; provided with a circular or horseshoeshaped crown of ciliated tentacles; individual animals small, usually microscopic.

Phylum Molluscoidea; Class Bryozoa (Polyzoa)

22 (21). Not colonial, nor microscopic, exoskeleton in the form of a calcareous shell of one or more pieces, or absent. 23

23 (24). With definite heads, sometimes bearing tentacles armed with suckers; shell, if present, not bivalve. **Phylum Mollusca**

24 (23). Without definite heads; shell bivalve. 25

25 (26). Halves of the shell dorsal and ventral in position; nearly always fastened by a stalk protruding between the valves at their hinge.

Phylum Molluscoidea; Class Brachiopoda

26 (25). Halves of the shell lateral; never with such a stalk, although sometimes sessile. **Phylum Mollusca**

27 (15). Body divided into segments, or with segmentally arranged bristles or internal organs, or with segmented appendages. 28

28 (33). Without jointed appendages; wormlike. 29

29 (30). Remarkably elongated, flat, tapelike worms, without mouth, anus, or digestive tract; head and anterior segments very small; segments very numerous, increasing greatly in size posteriorly, where they drop off when ripe; always internal parasites. **Phylum Platyhelminthes**

30 (29). Not tapelike; with anus, mouth, and digestive tract; segments decreasing in size posteriorly or of the same size throughout. 31

31 (32). Segments numerous, usually decreasing in size posteriorly; without tracheae, tracheal gills, or spiracles; never with definitely differentiated chitinized heads; often with segmental bristles, but these never in large tufts at one end of the body.

Phylum Annelida

32 (31). Segments few in number, not exceeding eleven or twelve; not markedly decreasing in size posteriorly; with tracheal tubes, tracheal gills, or spiracles; often separate hard chitinized heads; and may have bristles in tufts at one end.

Phylum Arthropoda (insect larvae)

33 (28). With jointed appendages, at least on the anterior segments of the body. **Phylum Arthropoda**

34 (12, 37). Wall of the pharynx pierced with gill slits; without an internal cartilaginous or bony skeleton. 35

35 (36). Small, fishlike forms; sides of the body marked with zigzag muscle segments; mouth without jaws, in the center of a rounded hood.

Phylum Chordata; Subphylum Cephalochorda

36 (35). Inclosed in a saclike gelatinous or tough coat, which has two openings to

the outside, for the ingress and egress of water; sessile, solitary or colonial (the latter quite small), or free-floating, marine. **Phylum Chordata; Subphylum Tunicata**

37. With a cartilaginous or bony skull and vertebral column.
Phylum Chordata; Subphylum Vertebrata

Key to the Classes of the Principal Phyla

I. Phylum Protozoa

1 (9). With temporary or permanent extensions of the surface of the body, mostly for locomotor purposes. 2

2 (3,6). Extensions in the form of changing pseudopodia, blunt to long and threadlike. **Class Sarcodina (Rhizopoda)**

3 (2,6). Extensions as raylike, non-motile (or nearly so) projections. 4

4 (5). Rays with a terminal knob. **Class Suctoria**

5 (4). Rays pointed, without a knob. **Class Sarcodina**

6 (2,3). Extensions in the form of long or short, hairlike, very active processes. 7

7 (8). Hairlike processes short and numerous (cilia). **Class Ciliata**

8 (7). Hairlike processes long and few (one or two to many). **Class Flagellata**

9 (1). Without locomotor or other processes of the body in the adult state; parasitic. **Class Sporozoa**

II. Phylum Coelenterata

1 (4,7). Animals of the hydroid type; sessile. 2

2 (3). Nearly always colonial; body of the hydroids on the end of slender stalks; without an esophagus; gastrovascular cavity, a simple sac, not divided by septa.
Class Hydrozoa

3 (2). Solitary or colonial; cylindrical or columnar, not divided into body and stalk; with an esophagus, and gastrovascular cavity divided into compartments by partitions. **Class Anthozoa**

4 (1,7). Animals of the medusa type, solitary, free-swimming. 5

5 (6). With a velum and of simple structure. **Class Hydrozoa**

6 (5). Without a velum, and more complex in structure, usually with highly branching gastrovascular canals. **Class Scyphozoa**

7 (1,4). Complex floating colonies containing both hydroid and medusa types of individuals. **Class Hydrozoa; Order Siphonophora**

III. Phylum Platyhelminthes

1 (4). With mouth and digestive tract; not especially elongated, nor divided into segments. 2

2 (3). Free-living, ciliated forms, without suckers. **Class Turbellaria**

3 (2). Parasitic, not ciliated; with at least one sucker, often more, often hooks in addition. **Class Trematoda**

4 (1). Without mouth or digestive tract; nearly always very long and tapelike, and divided into segments (not true segments); parasites. **Class Cestoda**

IV. Phylum Annelida

1 (4). Segmental bristles present; indefinite number of segments; no suckers. **Class Chaetopoda** 2

2 (3). Bristles numerous, generally on lateral outgrowths, the parapodia. **Subclass Polychaeta**

3 (2). Bristles few, set directly into the body wall; no parapodia. **Subclass Oligochaeta**

4 (1). Segmental bristles absent; limited number of segments; with an anal and an oral sucker. **Class Hirudinea**

V. Phylum Echinodermata

1 (8). With a well-developed skeleton of calcareous ossicles or plates; usually spiny; radial; no tentacles. 2

2 (3). Mostly sessile, attached by a stalk springing from the aboral surface; if free, moving on the aboral surface. **Class Crinoidea**

3 (2). Not sessile, nor stalked; moving on the oral surface. 4

4 (7). Star-shaped, five to many rays. 5

5 (6). Rays slender, sharply marked off from the disk. **Class Ophiuroidea**

6 (5). Rays broad, not sharply separated from the disk. **Class Asteroidea**

7 (4). Not star-shaped; spherical, oval, or flattened disks; very spiny. **Class Echinoidea**

8 (1). Skeleton rudimentary; animals with leathery and tough body walls; elongated, even wormlike, with a tendency to bilaterality; commonly with branched tentacles around the mouth. **Class Holothuroidea**

VI. Phylum Mollusca

1 (2). With arms provided with cuplike suckers; shell apparently absent, or, if present, spiral and divided into chambers. **Class Cephalopoda**

2 (1). Without such sucker-bearing arms; shell never divided into chambers, sometimes absent. 3

3 (4, 5). Shell bivalve. **Class Pelecypoda**

4 (3, 5). Shell of eight pieces. **Class Amphineura**

5 (3, 4). Shell univalve or absent. 6

6 (7). Shell shaped like an elephant's tusk. **Class Scaphopoda**

7 (6). Shell generally spirally coiled, sometimes cap-shaped or conical, sometimes absent. **Class Gasteropoda**

VII. Phylum Arthropoda

1 (2, 5). With two pairs of antennae in front of the mouth; generally covered with a hard exoskeleton; breathing by means of gills; usually with numerous appendages; aquatic or in damp places. **Class Crustacea**

2 (1, 5). With one pair of antennae; breathing by means of tracheae. 3

3 (4). Elongated, wormlike, with one or two pairs of jointed walking legs on each segment of the body. **Class Myriopoda**

4 (3). With only three pairs of jointed walking legs (larval forms of this class are provided for in the key to phyla). **Class Insecta**

5 (1, 2). Without antennae; four pairs of walking legs (in some cases appearing as six pairs through leglike condition of mouth parts). **Class Arachnida**

VIII. Subphylum Vertebrata

1 (4). Fishlike animals, living in water and breathing by means of gills; without lungs or limbs. **2**

2 (3). Without jaws or paired fins. **Class Cyclostomata**

3 (2). With jaws and paired fins. **Class Pisces**

4 (1). Not fishlike; nearly always with lungs (sometimes gills in addition); paired appendages in the form of limbs or absent. **5**

5 (6). Skin naked and slimy; never marine. **Class Amphibia**

6 (5). Skin provided with an exoskeleton consisting of hairs, scales, or feathers (if apparently absent, large marine forms). **7**

7 (8, 9). Exoskeleton in the form of scales, never with hair or feathers. **Class Reptilia**

8 (7, 9). Exoskeleton in the form of feathers and scales. **Class Aves**

9 (7, 8). Exoskeleton in the form of hairs (sometimes apparently absent, sometimes scales present also); always nourishing the young with milk. **Class Mammalia**

APPENDIX: PRACTICAL SUGGESTIONS

1. **Killing frogs.**—Frogs may be readily killed by placing them in a small, tightly closed container with a wad of cotton (size of the closed fist) soaked in ether or chloroform. Leave for 15 to 20 minutes after the animals become unconscious.

2. **Process of pithing.**—Grasp the frog firmly in the left hand and bend the head down by placing the forefinger across the snout. With a blunt instrument feel for the depression between the posterior end of the skull and the first vertebra (it is located at about the level of the fore limbs). Cut through the skin at this place with a fine scissors and press firmly on the depression with the blunt end of the instrument until the instrument breaks through the muscles into a cavity, the cavity of the skull. Then thrust the instrument forward into the brain moving it about so as to mash the brain thoroughly. If desired to pith the cord also, withdraw the instrument and thrust it in again backward down the full length of the neural canal pushing it in and out several times to mash the cord thoroughly. Always use a blunted instrument for pithing; never try to do it with a needle. A blunted hatpin or steel knitting needle or one prong of a long straight hairpin are good instruments for the purpose. If the pithing is properly done spontaneous movements cease. If the animal moves afterward, the instrument was probably thrust into the wrong place. When trying the feat for the first time one is likely to press too hard and to break into the mouth cavity instead of into the cranial cavity.

3. **Supplies for students.**—Each student should be provided with a jar having a tight cover and filled with 4 per cent formaldehyde (40 c.c. commercial formaldehyde plus 960 c.c. water); one or two watch glasses; one or two finger bowls with glass plates for covers; and a wax-bottomed dissecting pan. The jar should be large enough to receive comfortably the animals used in the course. Candy jars have been found cheap and convenient. Wax-bottomed dissecting pans can be purchased from dealers in biological supplies but can also be made, although in those made in ordinary pans, the wax bottoms frequently come loose. The following formula for the wax for dissecting pans has been kindly supplied by the General Biological Supply House: parowax, 75 parts; beeswax, 10 parts; cerosin, 15 parts. Blacken with lampblack. Use low granite ware pans about 9 by 12 inches and 1½ inches deep. Fill to a depth of 1½ inches with the wax.

4. **Chemicals required.**—The following solutions and solids are required in the course; they are required in the order named:

a) Dilute acetic acid for the wiping reflex, about 10 per cent: 10 c.c. of pure glacial acetic acid plus 90 c.c. of distilled water.

b) Physiological salt solution: dissolve 6½ grams of sodium chloride in a liter of distilled water.

c) Powdered carmine.

d) Artificial gastric juice: 1 to 2 grams of commercial pepsin (see list of dealers at end), in 100 c.c. of 0.4 per cent hydrochloric acid.

e) 0.4 per cent hydrochloric acid: 4 c.c. of pure concentrated hydrochloric acid plus 996 c.c. of distilled water.

f) Neutral litmus solution can be made by boiling litmus paper in water, but should preferably be purchased from dealers.

g) Pancreatic lipase: dissolve 1 to 2 grams of commercial pancreatin, in 100 c.c. distilled water. Add enough sodium carbonate to render barely alkaline with litmus.

h) Starch paste: stir up one gram of starch (corn starch or any commercial product) in a small amount of cold water until evenly suspended and then pour slowly while stirring into 100 c.c. of actively boiling distilled water (the water must be boiling). Allow to boil for a few minutes. Dilute about ten times for use.

i) Iodine solution. Dissolve 1 to 2 grams of potassium iodide in 100 c.c. distilled water and add enough iodine crystals to give a brown color.

j) Glucose solution: 4 or 5 grams of glucose in a liter of distilled water.

k) Fehling's solution consists of two solutions, not to be mixed until about to be used. First solution: 34.65 grams of copper sulphate made up to 500 c.c. with distilled water. Second solution: 125 grams of potassium hydroxide and 173 grams of Rochelle salt (potassium sodium tartrate) made up to 500 c.c. with distilled water. Keep separately in rubber-stoppered bottles. Just before using mix in equal quantities and shake until the blue precipitate formed is completely dissolved yielding a deep blue, clear solution. It is preferable that the mixing of the solutions be done by the instructor rather than by the students. Only enough of the mixture should be prepared as will be used during the laboratory period as the mixture does not keep well.

l) Saturated solution of barium hydroxide or lime water. Add an excess of barium hydroxide to distilled water, shake thoroughly at intervals. Add distilled water to unslaked lime, let stand for some time, and pour off the supernatant fluid. To keep both of these solutions free from carbonate, place in bottles with little air space, and stopper with a rubber cork pierced by two holes. Through one hole pass a siphon tube. Through the other pass a short glass tube the outer end of which connects with a calcium chloride tube (a test tube with a hole punched through the bottom will do) filled with soda lime. This arrangement necessitates that air passing in when any of the solution is siphoned off must pass over the soda lime which withdraws all carbon dioxide from it.

m) Aceto-carmine stain (Schneider's): to boiling 45 per cent glacial acetic acid (45 c.c. pure glacial acetic acid plus 55 c.c. distilled water) add powdered carmine until no more will dissolve and filter.

n) Carbon suspension for feeding Paramecium: get solid carbon sticks from dealers in photographic or artists' supplies, and rub one in a small amount of tap water until a moderately black suspension is obtained. Some samples of India ink are also usuable; others are toxic. For other solutions mentioned with regard to Paramecium see below under chapter viii.

o) Picro-acetic acid: 1 c.c. of pure glacial acetic acid, 99 c.c. of distilled water; saturate the solution with picric acid crystals.

p) Common salt.

A small bottle of each of these solutions should be placed on each table in the

laboratory to avoid confusion. Needless to say, starch, sugar, and enzyme solutions will not keep and must be made up fresh shortly before needed.

In addition to the above the instructor will require:

Ether or chloroform.

An egg and milk or cream for the work on digestion in chapter ii.

Chloral hydrate solution for macerating tissues of the frog. To 5 grams of chloral hydrate, add enough physiological salt solution to make 100 c.c.

5. Chapter i: General Survey.—Freshly killed frogs are desirable for this work as they are more agreeable to dissect, more pliable, and the organs appear in their natural colors. They may be kept for two or three days on ice. After that it will be necessary to put them in 4 per cent formaldehyde. If they are to be put into formalin before the interior is dissected care should be taken that only the skin is slit or at least that any opening through the body wall is very small in order to prevent protrusion and distortion of the viscera.

6. Chapter ii: General Physiology.—All of these experiments have been tried repeatedly and have never been known to fail. No frog should be given out that is not properly pithed as it will create a disturbance. The laboratory instructor should familiarize himself thoroughly with the process of pithing. There is seldom any difficulty in pithing the right place if a blunted instrument is used. Students should be warned to keep the frog moist. Dried preparations of enzymes furnished by drug houses (see list of dealers at the end) have always been found reliable. A small percentage of human beings have no ptyalin in their saliva. To demonstrate the circulation of the blood in the frog, the animal may be pithed in the brain but preferably should not be pithed. It can usually be kept quiet by wrapping it up in a moist towel. Obtain a sheet of cork, cut a round hole in it, pin the web of the hind foot across the hole, and place the hole over the opening in the stage of the microscope. Directions for the cultivation of Drosophila are given below under chapter vii.

7. Chapter iii: General histology.—This work always proves rather difficult chiefly because students use too large pieces. Verbal instructions as to the method of procedure will probably be more effective than those written in the outline. Shed epidermis of the frog will be found in the water in which frogs have been kept for a short time. To prepare tissues from the intestine proceed as follows. Pith a frog. Cut out the small intestine, slit it open, and wash it in physiological salt solution. For columnar epithelium put it into 5 per cent chloral hydrate for twelve to eighteen hours. The epithelial cells can then be readily scraped off. For smooth muscle scrape off the mucosa and place the rest, consisting of the muscular coats, into 5 per cent chloral hydrate for 1 to 2 days. It can then be teased readily. Connective tissue is always best obtained from a frog which has been preserved in formalin; and striated muscle is usually better from such a source unless the fresh muscle is carefully handled. Fresh slices of cartilage are very much better than any permanent preparations as the latter are nearly always badly shrunken; fresh cartilage is readily secured from the ends of the long limb bones of the frog with a sharp razor. Fresh blood may be obtained by cutting off the toes of a pithed frog and pressing the bleeding ends of the toes against a slide. It is very necessary that the physiological salt solution used in mounting fresh blood be of exactly the

same osmotic pressure as the blood, otherwise the blood corpuscles will become distorted. The myelin sheaths of fresh nerves usually undergo distortion producing artificial appearances which are commonly mistaken by the inexperienced for nodes of Ranvier. Students almost always have trouble with Golgi preparations of nerve cells often mistaking meaningless deposits of silver on the sections for nerve cells. Since writing the first edition of this manual the carmine preparations of the motor cells of the spinal cord of mammals have been found the most satisfactory material for the study of nerve cells by elementary students. The method of making these preparations emanates from G. H. Houser, *Journal of Applied Microscopy*, IV (1901), 1564. The method is as follows. Obtain a perfectly fresh length of the lumbar cord of the ox (or other mammal). Split lengthwise and scoop out from the ventral columns of the gray matter quite small pieces. Place these at once in Beale's carmine and stain about six weeks. Then put for a few days into glycerin diluted half with water and slightly acidulated with acetic acid. Transfer to pure glycerin in which the material may be left indefinitely. Whenever desired place a bit of the material on a slide in a drop of glycerin, put a circular cover glass on it and press straight down firmly mashing the tissue out into a thin film. Avoid rotating the cover glass. For permanent mounts replace the glycerin with glycerin jelly (for formula see section on embryology) and ring the cover glass with cement or varnish. Balsam mounts may also be made. Float the cover glass used in flattening the tissue off with 95 per cent alcohol. Dehydrate tissue in 95 per cent alcohol, two changes, 30 minutes each, absolute alcohol, two changes, 30 minutes each, clearer (xylol, wintergreen, etc.), 30 minutes. Mount in balsam.

Formula of Beale's carmine:

Carmine	1 gram
Ammonia (pure) . . .	3 c.c.
Pure glycerin . . .	96 c.c.
Distilled water . . .	96 c.c.
Alcohol, 95 per cent . .	24 c.c.

Add part of the water to the ammonia and dissolve the carmine in this with the aid of gentle heat. Let stand in an open dish for an hour until the ammonia has almost evaporated. Then add the other ingredients.

8. Chapter vi: Embryology.—If living amphibian eggs are not available, material preserved in formalin may be used. Strong light shining on the specimens is necessary in order to see the surface structures of whole amphibian embryos. Excellent permanent mounts of halves of early amphibian embryos can be made as follows. Use slides provided with cells or make such a cell by sticking hard rubber rings to the slide with balsam. Section the embryos in half with a sharp razor and mount in the cell in glycerin jelly. Ring the edge of the cover glass (the jelly should not extend quite to the edge) with cement or varnish. To make glycerin jelly, dissolve six grams of best gelatin in 42 c.c. of water, with the aid of gentle heat. Add about 5 c.c. of white of egg and heat (not over 75° C.) for half an hour. The purpose of this is to collect all dust in the gelatin in the egg white which precipitates. Filter through moist hot fine flannel and add 50 c.c. of glycerin and 2 grams of carbolic acid crystals. Warm for 10 or 15

minutes stirring continuously until homogeneous. Each time the jelly is to be used it must be liquefied by placing the bottle in warm water. This method is taken from Guyer, *Animal Micrology*, 1917, University of Chicago Press.

Living eggs of frogs are obtainable in the spring from March through May. They are found in ponds and marshes attached to the vegetation below the surface or floating on the surface in frothy masses. They develop well under laboratory conditions. After emergence the tadpoles must be fed. If one desires to observe the entire development through metamorphosis, the eggs of a quickly developing species should be selected. For identification of frog eggs and tadpoles, the pamphlet by A. H. Wright entitled "Frogs: Their Natural History and Utilization" is invaluable. It is obtainable from the Superintendent of Documents, Washington, D. C., for 15 cents. See also his article in the *American Naturalist*, Vol. LVIII, 1924. To rear tadpoles they should be provided first of all with green food. Mats of green algae are best for this purpose but in lieu of anything else, alfalfa hay can be used. In addition to the green food, hard-boiled yolks of eggs mixed into balls with wheat flour and small bits of fresh liver or fresh bodies of dead animals should be added. The water in which the tadpoles are kept should be shallow and must not be allowed to become foul. At the time of emergence of the forelimbs, objects on which the young frogs can climb for air should be placed in the dishes and the amount of water reduced to just enough to cover the tadpoles. The metamorphosing tadpoles are very likely to drown if these precautions are not observed. The dish should also be covered at this time otherwise they are likely to jump out. The young frogs can be fed if desired on small insects such as Drosophila.

9. **Chapter vii: Mendel's law.**—The following method is now used for culturing Drosophila (see Bridges, *American Naturalist*, LV (1921), 54). Use 4-, 8-, or 16-ounce wide-mouthed bottles or half-pint or pint milk bottles, depending on quantity of Drosophila desired. These bottles should be sterilized in advance in boiling water or preferably in an autoclave. Use thoroughly ripe or over-ripe bananas. Discard the skins. To each 100 grams of banana take 100 c.c. of water and 2 grams of agar-agar. Add the agar-agar to the water and heat until dissolved. Press the banana through a sieve and stir this banana mash into the hot agar, mixing thoroughly. Let boil two minutes stirring constantly. Pour the hot mixture into the bottles to the depth of about an inch. Place in each bottle in contact with the medium a piece of absorbent paper toweling folded twice, the dimensions after folding to be about 2 by 3 inches. Sprinkle the surface of the medium lightly with ground up dry yeast and stopper the bottle with cotton. Add Drosophila within 24 hours. They may be added as soon as the bottles cool. The medium will not keep unless Drosophila are present. Living Drosophila are sold by some of the dealers listed at the end of this chapter.

10. **Chapter viii: Protozoa.**—

a) To obtain Amoeba. Cook some vigorous sprays of water weeds such as Elodea or Ceratophyllum for fifteen minutes in boiling water. Place the cooked weeds in jars of water adding about one spray per liter of water. After one week add per liter 10 grains of wheat which have been boiled for several minutes and a few small bits of raw meat (or better, the crushed bodies of small aquatic animals). At this time add a small quantity of pond material that may be supposed to contain Amoeba. This inoculation

with materials believed to contain Amoeba should be repeated at intervals if Amoeba does not appear. The weeds should decay producing a greenish layer on the bottom which should be examined for Amoeba at intervals beginning one week after inoculating. The culture must remain clear and should show no visible signs of fermentation. If it does too much food material has been added and some of it should be removed. Material suitable for inoculating such cultures should consist of about a tablespoonful of decaying material from a pond. Cultures of this kind will furnish Amoeba for a long time once they develop Amoeba and if a desirable species of Amoeba is found it can be continued indefinitely by transferring from time to time to new cultures made as directed. Amoeba will be found only on the bottom of such cultures.

b) To obtain Euglena. Collect from ponds any greenish looking material as surface scums and mats of algae. Place in dishes on the window sill adding about twice as much water as there is solid material. After two or three days examine drops taken from the surface of the water at the edge nearest the light. Such cultures are good for only a few days. To cultivate Euglena boil grains of ordinary white (polished) rice for 15 to 30 minutes. Add several grains of this rice to each 500 c.c. of water and after this has stood for several days inoculate with Euglena.

c) To obtain Paramecium. Collect water weeds, decaying plants, and bottom débris from a pond and place in jars, adding just enough water to cover the material. After a few days Paramecium will be found in such cultures and will increase up to a certain time when they will begin to diminish. When this happens they can be caused to increase again by adding a few crumbs of stale white bread to the culture. To cultivate Paramecium continuously boil some wheat grains for 15 minutes and add to jars of water so that half of the bottom of the jar is loosely covered with a single layer of grains. After this has stood for one week add Paramecium to it. Each such culture will last for three or four weeks when a new jar should be prepared and seeded with Paramecium.

d) To obtain a variety of Protozoa. Collect plants, débris, etc., from a pond and place in jars varying the amount of water to the amount of solid material. In some jars have much water to a small amount of pond material, in others much material and little water, and in others half water and half material. A variety of Protozoa should appear in such cultures.

e) To quiet Paramecium. Various viscous media have been used to make Paramecium stand still. Of these the best is Chondrus, a marine alga commonly known as Irish moss. It can be obtained from the Whitfield Food Products Co., 215 North State Street, Chicago. First wash it several hours in running water to remove sea salts dried on it. Then boil in water. It gives off something into the water which when cool sets into a jelly. The proper amount of this added to a drop of Paramecium on a slide will stop the animals from moving about without injuring or distorting them.

The formaldehyde method is also effective. Add one drop of formalin to 100 c.c. of water and test it on Paramecium. If too strong dilute until a strength is found which seems to have no effect immediately but which in a few minutes causes the animals to slow down gradually. The solution may be used as directed in the manual or a

drop of the solution may be placed alongside the edge of the cover glass. In the latter case a stronger solution will be required.

Bills (*Biological Bulletin,* XLII, 1) recommends normal propyl or isopropyl alcohol for anaesthetizing Paramecium for class study. The proper concentration is 2–3 per cent of the alcohol (2 to 3 parts of the alcohol to 97 to 98 parts of water by volume). But as the Paramecium is already in water a stronger solution of the alcohol should be used to start with as it becomes diluted on addition to the Paramecium. A good procedure is to take one volume of Paramecium suspension and add an equal volume of 4–6 per cent of the alcohol to this. Or to a drop of Paramecium on a slide add one drop of 4–6 per cent alcohol. About 30 minutes are required to anaesthetize the animals. Bills considers this method superior to all other methods of quieting Paramecium. It does not succeed very well with other Protozoa.

11. Chapter ix: Coelenterata.—To obtain Hydra, collect aquatic plants from clear ponds or bays in streams. Place the plants in jars using a large amount of water to a small quantity of the plants. Examine for Hydra after a day or two, looking on the plants, sides of the jar, and surface film. Hydra can readily be cultivated in the laboratory if sufficient food is provided. The most convenient food consists of Entomostraca (Daphnia and other daphnids). To grow Entomostraca, cook some water weeds and place the cooked weeds in jars or pans of water. After a week add Entomostraca. They can be secured by running a net of finely meshed cloth through the open water of a pond. Add pieces of raw meat or bodies of dead animals to the entomostracan culture from time to time. It must not be allowed to ferment too rapidly. Feed Hydra every day or two by adding Entomostraca to the Hydra culture.

The work on the cellular structure of the living animal is generally difficult for students to carry out and may be profitably omitted. So also the experiments with the living animal which frequently fail because students handle the animals too roughly.

Sexual specimens of Hydra can usually be obtained from out-of-doors in the fall of the year and sometimes appear in laboratory cultures. To make Hydra bud feed plenty of Entomostraca for several days.

12. Chapter x: Platyhelminthes.—Planarians may be found in the following places: on the under surface of stones along the borders of streams and ponds; on the water weeds in ponds; and in springs. They can usually be obtained by letting a lot of pond weeds decay in a small amount of water whereupon the planarians come to the top and can be picked out. Species that live in springs can be caught by placing pieces of raw beef along the edges of the current. Planarians can readily be kept in the laboratory in pans or dishes in which the water is frequently changed. They should be fed two or three times a week. Most species will feed on pieces of raw liver which should be strewn on the bottom of the pan. Species that will not eat meat should be tried with crushed amphipods or isopods or pieces of earthworms, clams, etc. After feeding remove the meat and rinse the pan thoroughly. The digestive tract of Planaria will always stand out plainly if the anaesthetized animals are pressed out with a cover glass or a piece of thin slide. However, it can be made very conspicuous by feeding the animals on blood clot shortly before they are to be used. Catch the blood from any vertebrate (must not have been anaesthetized!) into a clean beaker and let stand quietly until the

blood clots. Cut the clot into small pieces, rinse in water, and place the pieces in the dishes with Planaria, which should have been starved for one to two weeks. For method of making permanent whole mounts of such worms fed on blood see *Transactions of the American Microscopical Society* for January, 1924, p. 70. For class demonstration of the feeding of planarians, use only worms that have been starved at least a week.

13. **Chapter xii.**—The dissection of the reproductive system should ordinarily be omitted. Living earthworms can be obtained at all times of the year from Paul A. Webb, Meadville, Pennsylvania. They can be kept for some time in damp earth, but must not be crowded. They will eat potato parings, lettuce, cabbage, etc.

14. **Chapter xiii.**—Pond snails can be obtained from any pond; gilled snails generally inhabit streams where they are found on the under side of stones, or the sandy bottoms of ponds and small lakes. They are readily kept in the laboratory if supplied with pond weeds for food. They also feed readily on pieces of meat. Land snails occur in moist woods under leaves and logs. For laboratory study, preserved specimens of the common land snails Helix or Polygyra are satisfactory. Preserved pond snails such as Lymnaea or Physa can be used but are rather small. Fresh water clams occur in gravelly or sandy beds of streams or small shallow lakes. They are readily kept in the laboratory in aquaria provided with sand banks. It is generally not necessary to try to feed them.

15. **Chapter xiv.**—Crayfish are readily obtained from ponds and streams where they will be found among the vegetation or under stones. Keep them in rather shallow water with vegetation. They are best fed from time to time on bits of raw meat or bodies of recently killed animals.

16. **Chapter xv.**—The order in which this chapter is written assumes that the muscular system will not be studied in any detail and that an injected frog will be supplied for the circulatory system. In case a single (injected) frog is to serve for the entire dissection, the order should be changed. The muscular system should be dissected first if this system is to be studied. The dissection of the circulatory system should precede the digestive, urogenital, and respiratory systems. To inject a frog, etherize it, cut off the tip of the ventricle, and thrust the cannula of the injection syringe up into the conus arteriosus. Avoid too strong pressure. All of the arteries and frequently the postcaval vein are injected by this procedure. It is not necessary to tie the cannula in the conus or tie up the conus after withdrawing the cannula; simply hold the cannula in with the fingers during the injection. One formula for the injection fluids is the following: 100 c.c. of water, 20 c.c. of glycerin, 20 c.c. of formaldehyde, 85 grams of corn starch, sufficient coloring matter (mercuric sulphide, lead chromate, or Prussian blue) to give a deep color. Stir thoroughly before using. For gelatin injection masses, see Guyer, *Animal Micrology*, University of Chicago Press. Veins are best studied on freshly etherized animals, although they are fairly satisfactory on injected specimens if injected shortly before using. It is generally impossible to work out the veins satisfactorily on specimens that have been preserved for any length of time. It is feasible to let the students study the venous system on freshly killed frogs, and then to inject the arterial systems on the same animals after the study of the veins is finished

After the frogs are injected a piece of the skull should be removed and the frogs placed in about 8 per cent formaldehyde to harden the brain and vessels.

17. Dealers.—Living Protozoa, Hydra, Planaria, and microscopic preparations of these forms, of mitosis, etc., can be obtained from Power and Powers, Station A, Lincoln, Nebraska. Living and preserved materials and microscopic preparations can be obtained from a number of dealers, e.g.: General Biological Supply House, 761–63, East 69th Place, Chicago; Denoyer-Geppert Co., 5235–57, Ravenswood Avenue, Chicago; New York Biological Supply Co., 34, Union Square, New York; Supply Department, Marine Biological Laboratory, Woods Hole, Massachusetts; Michigan Biological Supply Co., 324, S. State Street, Ann Arbor, Michigan; Southern Biological Supply Co., Natural History Building, New Orleans, Louisiana; Pacific Biological Laboratories, Pacific Grove, California; Ward's Natural Science Establishment, Rochester, New York; Kny-Scheerer Corporation, 17th Street and 7th Avenue, New York. Living earthworms are sold at all seasons of the year by Paul A. Webb, Meadville, Pennsylvania. Pepsin, pancreatin, and similar preparations can be obtained from Armour and Co., Chicago, or Parke, Davis, and Co., Detroit, Michigan, or Merck and Co., New York.

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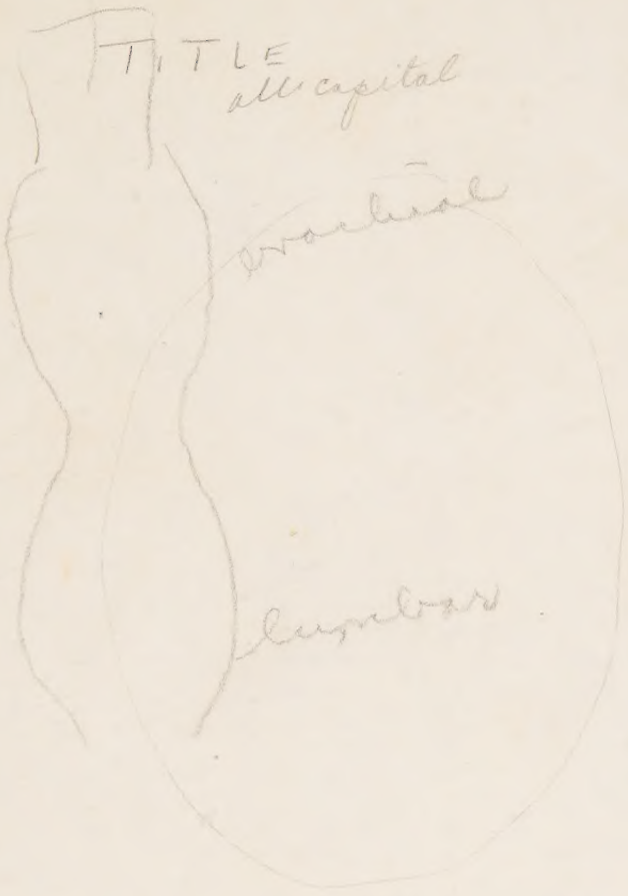
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